

Dissertation submitted as a partial fulfilment of the requirements
for the degree Doctor of Natural Sciences (Dr. rer. nat.)
at the Faculty of Biology and Chemistry, University of Bremen

Self-recruitment in anemonefish and the impact of marine ornamental fishery in Spermonde Archipelago, Indonesia: implications for management and conservation

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Bremen, March 2012

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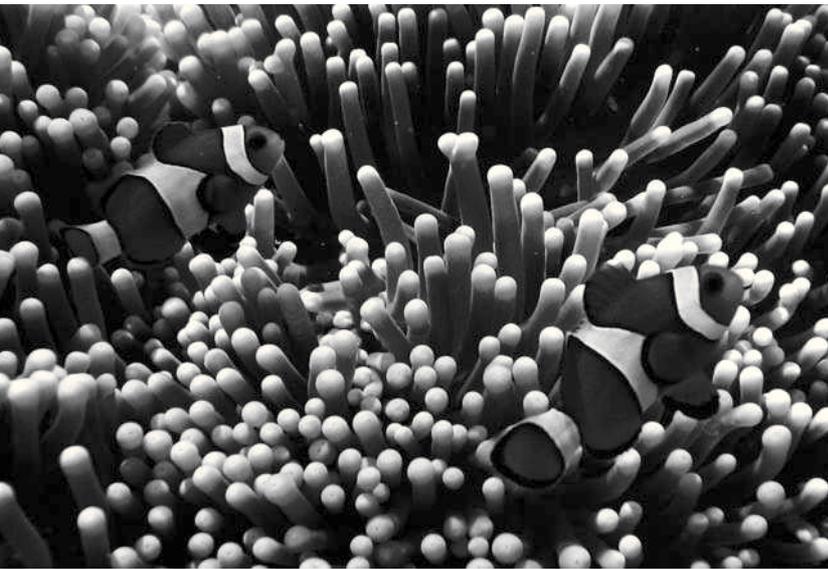
Bremen, March 2012

to the loved ones
Amnah and our children, Raihan and Khailila



◀ Photo on cover: A juvenile of *Amphiprion ocellaris* on its host anemone *Heteractis magnifica* at the Spermonde Archipelago

A pair of *Amphiprion ocellaris* on its host anemone *Heteractis magnifica* at the Spermonde Archipelago ▶



"The worst thing that will probably happen – in fact is already well underway – is not energy depletion, economic collapse, conventional war, or the expansion of totalitarian governments. As terrible as these catastrophes would be for us, they can be repaired in a few generations. The one process now going on that will take millions of years to correct is loss of genetic and species diversity by the destruction of natural habitats. This is the folly our descendants are least likely to forgive us."

EDWARD O. WILSON (1984)

"The ocean is not a safe place for fish"
said Marlin to Nemo.

FINDING NEMO (2003)





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Summary

Indonesia's coral reefs have been considered as the centre of coral triangle due to high number of coral and reef fish species. While these reef resources sustain the livelihoods of millions of people, they are also among the most threatened by anthropogenic activities, including pressure from marine ornamental trade. Spermonde Archipelago is one of the main collection sites for marine ornamental trade in Indonesia. Many coral reef fishes are currently collected for the aquarium trade, of which the clown anemonefish "Nemo" *Amphiprion ocellaris* is the most popular species. The trade is ongoing, unfortunately the total catch and the potential impact of high exploitation rates are not known. Additionally, no proper management and conservation efforts exist in Indonesia to protect this species. This situation will potentially lead to the degradation of this focal species and contribute to overall marine biodiversity degradation. Thus, a proper management, including the implementation and effective enforcement of marine protected areas (MPAs), is needed to avoid overexploitation and depletion of this commercially harvested fish species. The connectivity of populations and the extent of self-recruitment are important aspects for the management of living resources and designing and improving of MPAs.

The aim of this thesis is divided into two parts of studies as the following.

First a study on the status and the impact of ornamental fishery on anemonefish at population and genetic level was conducted. Therefore interviews with fishermen and middlemen were conducted to reveal current trade status. Field surveys were carried out at 28 reef sites within Spermonde Archipelago to assess the abundance of *A. ocellaris* and its host anemones. The total length for all *A. ocellaris* individuals at five reef sites at Barrang Lompo Island (historically high exploited reefs) and four at Samalona Island (relatively low exploited reefs) was measured. Polymorphic microsatellite loci were used to investigate genetic diversity of this species in Barrang Lompo and Samalona. The results were compared to the fish population densities.

This study revealed that more than 25,000 specimens of *A. ocellaris* (93 % of the total ornamental fish trade) were traded in Balang Lompo over a three-month

period in 2005/2006 and more than 10,000 specimens (83 %) were recorded by middlemen on Barrang Lompo in 2008. About 650 anemones were traded by middlemen over a four-month period in 2005 in Karanrang and Barrang Lompo, while during a three-month period in 2008 more than 7,400 anemones were recorded by middlemen on Barrang Lompo. The yearly amount of traded by middlemen on these islands is estimated to about 140,000 specimens *A. ocellaris* and more than 31,000 anemones. Both *A. ocellaris* and sea anemone densities ($p < 0.01$) were significantly lower at reefs with a high exploitation than at reefs with a low exploitation. In addition the size of *A. ocellaris* individuals was significantly smaller in Barrang Lompo than in Samalona ($p < 0.01$). These findings revealed a considerable negative impact of marine ornamental fishery on the target populations.

The investigation of the polymorphic microsatellite loci in Barrang Lompo and Samalona revealed a high diversity within both populations (mean HE and D ~ 0.80). Despite this a weak but significant genetic difference between the two populations was found (0.065 ± 0.048 , $p = 0.01$). The heterozygote deficit at Barrang Lompo was higher than at Samalona, but no obvious difference between the island populations was found ($p > 0.05$). A low inbreeding coefficient of both islands was estimated (mean 0.067 ± 0.125 at Barrang Lompo and mean 0.042 ± 0.123 at Samalona). The genetic diversity (private alleles and allelic richness) in Samalona was 4% significantly higher than in Barrang Lompo. The allelic richness was positively correlated with the fish density ($p < 0.05$). These results indicated that the *A. ocellaris* collection at the archipelago caused loss of genetic diversity.

The second part of the thesis is a study on self-recruitment, genetic relatedness, and site fidelity in anemonefish. Polymorphic microsatellite loci facilitate these studies on two anemonefish species, the clown anemonefish *A. ocellaris* and the pink anemonefish *A. perideraion*.

The study revealed that the self-recruitment of *A. ocellaris* in Samalona (60.7 %) was higher than for *A. ocellaris* (47.4 %) and *A. perideraion* (44 %) in Barrang Lompo. The estimated self-recruitment for *A. ocellaris* in Samalona was much higher than the reported self-recruitment of the sibling species *A. percula*. This

means fish stays largely in place despite its pelagic larvae stage. The investigation of the genetic relatedness revealed a close relation between individuals within a group of *A. ocellaris* in Barrang Lompo. Conversely, unrelated individuals of *A. ocellaris* in Samalona and of *A. perideraion* in Barrang Lompo were observed.

Altogether, these results provide important insights how marine ornamental fishery has impacted the population of anemonefish and its host in Spermonde Archipelago. The fish populations at two islands valuated as low and high exploited in terms of fishery are found to differ in many fish biology parameters. High self-recruitment implies that single marine protected areas are not suitable as sources for the replenishment of exploited populations. For this task small MPAs, preferably on every island/reef, should be considered.

Zusammenfassung

Indonesiens Korallenriffe sind aufgrund der hohen Anzahl an verschiedenen Korallen- und Fischarten als das Zentrum des s.g. Korallendreiecks bezeichnet worden. Während diese Riff-Ressourcen einen großen Teil der Lebensgrundlage von Millionen von Menschen bilden, werden sie gleichzeitig durch anthropogene Aktivitäten, einschließlich des Drucks durch Zierfischhandel, bedroht. Das Spermonde Archipel ist eins der wichtigsten Fanggebiete für Zierfische in Indonesien. Viele Korallenriff-Fische werden derzeit für den Aquarienhandel gesammelt, von diesen ist der Clown-Anemonenfisch "Nemo" *Amphiprion ocellaris* die beliebteste Art. Während der Handel in vollem Gange ist, sind die gefangenen Mengen und die möglichen Auswirkungen der hohen Befischung leider nicht bekannt. Darüber hinaus gibt es keine angemessene Bewirtschaftungs- und Erhaltungsbemühungen in Indonesien, um diese Arten zu schützen. Diese Situation wird möglicherweise zur Schwächung dieser Schwerpunkt Art führen und dazu beitragen die marine Artenvielfalt insgesamt zu reduzieren. Daher ist eine ordnungsgemäße Verwaltung, einschließlich der Einrichtung und wirksamen Durchsetzung von Meeresschutzgebieten (MPAs), nötig, um Raubbau und Überfischung dieser kommerziell genutzten Fischarten zu vermeiden. Der genetische Zusammenhang zwischen Populationen und das Ausmaß der Selbstrekutierung sind wichtige Aspekte für die Bewirtschaftung der Bestände und die Gestaltung und Verbesserung der MPAs.

Das Ziel dieser Arbeit ist wie folgt in zwei Studien unterteilt.

Erstens wurden das Ausmaß und die Auswirkungen des Fangs von Clownfischen sowohl auf der Populations- als auch auf der genetischen Ebene untersucht. Dafür wurden Interviews mit Fischer und Zwischenhändlern geführt, um den aktuellen Zustand zu erheben. Feldstudien wurden an 28 Riffe innerhalb des Spermonde Archipelagos durchgeführt, um die Populationsgröße von *A. ocellaris* und seinen Wirtsanemonen zu beurteilen. Die Gesamtlänge für alle *A. ocellaris* Individuen an fünf Riffen der Insel Barrang Lompo (historisch intensiv genutzte Riffe) und vier der Insel Samalona (relativ gering genutzte Riffe) wurde gemessen. Polymorphe Mikrosatelliten-Loci wurden verwendet, um die

genetische Vielfalt dieser Art in Barrang Lompo und Samalona zu bestimmen und diese mit der Populationsdichte vergleichen zu können.

Diese Studie ergab, dass mehr als 25.000 Exemplare von *A. ocellaris* (93% des gesamten Zierfischhandels) in Balang Lompo über einen Zeitraum von drei Monaten in den Jahren 2005/2006 gehandelt wurden und mehr als 10.000 Proben (83%) wurden von Zwischenhändlern auf Barrang Lompo im Jahr 2008 erfasst. Rund 650 Anemonen wurden durch Zwischenhändler über einen Zeitraum von vier Monaten im Jahr 2005 auf Karanrang und Barrang Lompo gehandelt, während in einem Drei-Monats-Zeitraum im Jahr 2008 mehr als 7.400 Anemonen durch Zwischenhändler auf Barrang Lompo registriert wurden. Die jährliche Menge die durch Zwischenhändler auf diesen Inseln gehandelt wird, wird auf rund 140.000 Exemplare *A. ocellaris* und mehr als 31.000 Anemonen geschätzt. Sowohl die Dichte an *A. ocellaris* als auch an Seeanemonen ($p < 0,01$) waren signifikant niedriger an Riffen mit einer hohen Ausbeutung als an solchen mit einer niedrigen. Außerdem war die Größe der *A. ocellaris* Individuen deutlich kleiner in Barrang Lompo als in Samalona ($p < 0,01$). Diese Ergebnisse zeigten eine erhebliche, negative Auswirkung des Zierfischhandels auf die untersuchten Populationen.

Die Untersuchung der polymorphen Mikrosatelliten-Loci in Barrang Lompo und Samalona ergab eine große genetische Variation innerhalb beider Populationen (Mittelwert H_E und $D \sim 0,80$). Trotzdem wurde ein schwacher aber signifikanter genetische Unterschied zwischen den beiden Populationen gefunden ($0,065 \pm 0,048$, $p = 0,01$). Das heterozygote Defizit auf Barrang Lompo war höher als auf Samalona. Es konnte aber kein deutlicher Unterschied zwischen den Populationen gefunden werden ($p > 0,05$). Es wurde ein niedriger Inzuchtkoeffizient für beiden Inseln bestimmt (Mittelwert $0,067 \pm 0,125$ für Barrang Lompo und $0,042 \pm 0,123$ für Samalona). Die genetische Vielfalt (private Allele und Allel-Reichtum) war in Samalona 4% signifikant höher als in Barrang Lompo. Der Allel-Reichtum war positiv mit der Fisch Dichte ($p < 0,05$) korreliert. Diese Ergebnisse zeigten, dass die Befischung von *A. ocellaris* im Archipel zu einem Verlust an genetischer Vielfalt führt.

Der zweite Teil der vorliegenden Arbeit ist eine Studie über Selbstrekrutierung, genetische Verwandtschaft und Ortstreue bei Clownfischen. Hierzu wurden polymorphe Mikrosatelliten-Loci von zwei Arten, dem Clown Anemonenfisch *A. ocellaris* und dem Rosa Anemonefish *A. perideraion*, ausgewertet.

Die Untersuchung ergab, dass die Selbstrekrutierung von *A. ocellaris* in Samalona (60.7%) höher als bei *A. ocellaris* (47.4%) und *A. perideraion* (44%) in Barrang Lompo war. Die geschätzte Selbstrekrutierung für *A. ocellaris* in Samalona war viel höher als die gemeldete Selbstrekrutierung der Geschwisterart *A. percula*. Das bedeutet, dass die Fische trotz ihrer dispergierten Larven größtenteils ortstreu sind. Die Untersuchung der genetischen Verwandtschaft ergab eine nahe Verwandtschaft zwischen Individuen von *A. ocellaris* in Barrang Lompo. Dagegen wurden nicht verwandte Individuen von *A. ocellaris* in Samalona und von *A. perideraion* in Barrang Lompo gefunden.

Insgesamt ergeben diese Ergebnisse wichtige Einblicke in die Auswirkungen des Zierfischhandels auf die Populationen von Clownfischen und ihren Anemonen im Spermonde Archipel. Die Fischbestände an zwei Inseln, die jeweils als intensiv oder relativ gering genutzt eingeschätzt wurden, zeigten Unterschiede im Bezug auf viele Aspekte der Fischbiologie. Das hohe Maß an Selbstrekrutierung zeigt, dass einzelne Meeresschutzgebiete nicht ausreichend sind, um die Erholung der ausgebeuteten Bestände zu gewährleisten. Hierfür eignen sich kleine Schutzzonen an jeder Insel oder jedem Riff besser.

Rangkuman

Indonesia dan perairan di sekitarnya dinyatakan sebagai pusat keanekaragaman hayati dunia yang dikenal dengan Segitiga Terumbu Karang Dunia dengan jumlah jenis koral dan ikan yang sangat tinggi. Namun terumbu karang Indonesia termasuk yang paling terancam keberadaannya karena tingginya tekanan antropogenik. Hal ini karena ekosistem terumbu karang tersebut menjadi mata pencaharian bagi jutaan orang termasuk dari industri perikanan hias laut. Indonesia merupakan salah satu eksportir utama dalam perdagangan ikan hias laut dan Kepulauan Spermonde merupakan salah satu lokasi penangkapan utama di negara ini. Ikan anemon *Amphiprion ocellaris* merupakan spesies yang paling populer diantara banyaknya jenis ikan terumbu yang diperdagangkan. Perdagangan tetap terus berlanjut, akan tetapi jumlah tangkapan dan dampak dari penangkapan tersebut belum diketahui. Sebagai tambahan, sedikit upaya manajemen dan konservasi telah dibuat untuk mencegah penangkapan yang berlebihan. Situasi ini secara potensial dapat menyebabkan degradasi pada ikan target ini dan keanekaragaman hayati secara keseluruhan. Aspek penting yang perlu ditinjau untuk pengelolaan sumber daya hayati laut adalah sejauh mana rekrutmen-lokal dan konektivitas genetik dari suatu populasi.

Thesis ini dibagi menjadi dua bagian studi seperti yang dipaparkan berikut ini.

Bagian pertama dari thesis ini adalah studi tentang status dan dampak perikanan hias pada ikan anemon pada tingkat populasi dan tingkat genetik. Wawancara dilakukan dengan nelayan dan pengumpul untuk mendapatkan gambaran status terkini. Survei lapangan dilakukan pada 28 lokasi terumbu karang di perairan Kepulauan Spermonde untuk mendata kelimpahan *A. ocellaris* dan anemon inangnya. Pengukuran panjang total individu *A. ocellaris* dilakukan di lima lokasi terumbu di Pulau Barrang Lompo (terumbu yang dieksploitasi tinggi) dan empat lokasi di Pulau Samalona (terumbu yang dieksploitasi rendah). Lokus mikrosatelit polimorfik digunakan untuk menyelidiki keragaman genetik *A. ocellaris* di Barrang Lompo dan Samalona, dan membandingkannya dengan kepadatan populasi ikan.

Penelitian ini mengungkapkan bahwa lebih dari 25.000 spesimen *A. ocellaris* (93% dari total perdagangan ikan hias) yang diperdagangkan di Balang Lompo selama periode tiga bulan pada tahun 2005/2006 dan lebih dari 10.000 spesimen (83%) dicatat oleh pengumpul di Barrang Lompo pada tahun 2008. Sekitar 650 anemon diperdagangkan oleh pengumpul selama empat bulan pada tahun 2005 di Barrang Lompo dan Karanrang, sementara lebih dari 7.400 anemon dicatat oleh pengumpul Barrang Lompo selama periode tiga bulan di tahun 2008. Diperkirakan sekitar 140.000 spesimen *A. ocellaris* dan lebih dari 31.000 anemon untuk jumlah tahunan yang diperdagangkan oleh pengumpul di pulau-pulau ini. Kepadatan *A. ocellaris* dan anemon laut ($p < 0.01$) secara signifikan lebih rendah pada terumbu dengan eksploitasi tinggi (14 lokasi) daripada di terumbu dengan eksploitasi rendah (14 lokasi). Ukuran individu *A. ocellaris* secara signifikan lebih kecil di Barrang Lompo daripada di Samalona ($p < 0.01$). Temuan ini menunjukkan adanya indikasi dampak negatif yang cukup besar dari kegiatan perikanan ikan hias laut terhadap populasi ikan *A. ocellaris* dan inang anemonnya.

Investigasi lokus mikrosatelit polimorfik di Barrang Lompo dan Samalona menunjukkan keragaman genetik yang tinggi pada kedua populasi (rata-rata HE dan $D \sim 0,80$). Terlepas dari ini, ditemukan perbedaan genetik yang lemah tapi signifikan ($0,065 \pm 0,048$, $p = 0,01$) antara dua populasi. Heterozigot defisit di Barrang Lompo lebih tinggi dari pada Samalona, tetapi tidak ditemukan perbedaan jelas antara dua populasi pulau tersebut ($p > 0,05$). Koefisien inbreeding dari kedua pulau diperkirakan rendah ($0,067 \pm 0,125$ di Barrang Lompo dan $0,042 \pm 0,123$ di Samalona). Keragaman genetik (alel privat dan kekayaan alelik) di Samalona ditemukan sebanyak 4% signifikan lebih tinggi daripada di Barrang Lompo. Kekayaan alelik berkorelasi positif dengan kepadatan ikan ($p < 0,05$). Hasil ini menunjukkan bahwa *A. ocellaris* terindikasi terkena dampak dari aktivitas perdagangan yang menyebabkan hilangnya keanekaragaman genetik.

Bagian kedua dari thesis ini adalah studi rekrutmen-lokal, kekerabatan genetik dan situs fideliti pada ikan anemon. Lokus mikrosatelit polimorfik digunakan untuk memfasilitasi studi ini pada dua spesies ikan anemon, *A. ocellaris* dan *A. perideraion*.

Hasil studi ini menunjukkan rekrutmen-lokal *A. ocellaris* di Samalona (60.7%) lebih tinggi daripada Barrang Lompo untuk *A. ocellaris* (47.4%) dan *A. perideraion* (44%). Hasil estimasi lokal-rekrutment untuk *A. ocellaris* di Samalona jauh lebih tinggi daripada yang dilaporkan dari spesies kerabat terdekatnya *A. percula*. Hal ini menunjukkan bahwa ikan tetap tinggal ditempatnya dalam proporsi yang tinggi, terlepas dari daur hidup pelagisnya. Hasil investigasi kekerabatan genetik menunjukkan bahwa adanya kekerabatan dekat antara individu ikan di dalam satu koloni *A. ocellaris* di Barrang Lompo. Sebaliknya, diperkirakan tidak adanya kekerabatan dekat antara individu *A. ocellaris* di Samalona dan *A. perideraion* di Barrang Lompo.

Secara keseluruhan, hasil ini memberikan wawasan penting tentang dampak perikanan hias laut pada populasi ikan anemon dan inangnya. Populasi ikan pada kedua pulau, yang berbeda tingkat eksploitasinya, ditemukan berbeda pada banyak parameter biologi ikan. Nilai rekrutmen-lokal yang tinggi menyiratkan bahwa Daerah Perlindungan Laut (MPA) tunggal tidak cocok untuk sumber peremajaan ikan yang mengalami eksploitasi. MPA yang relatif kecil, misalnya di setiap pulau atau terumbu karang pulau, perlu dipertimbangkan sebagai upaya manajemen dan konservasi.

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List of publications

Publication 1

Title: Catching Nemo: impact of marine ornamental fishery on the population structure of the clown anemonefish *Amphiprion ocellaris* and its host anemones in Spermonde Archipelago, Indonesia

Authors: Hawis H. Madduppa, Karen von Juterzenka, Mohammad Syakir, and Marc Kochzius

Journal: Ocean and Coastal Management (submitted)

The idea to this study was developed by Hawis Madduppa and Marc Kochzius. Field sampling was done by Hawis Madduppa together with Agus Nuryanto and students from Marine Science Diving Club Hasanuddin University. The first part (socio-economic structure) of the paper was conducted by Mohammad Syakir. The data analysis was carried out independently by Hawis Madduppa. The manuscript was written by Hawis Madduppa with revisions and improvements by Marc Kochzius and Karen von Juterzenka.

Publication 2

Title: Fishery-induced loss of genetic diversity in the clown anemonefish *Amphiprion ocellaris* in island populations of the Spermonde Archipelago

Authors: Hawis H. Madduppa, Janne Timm, and Marc Kochzius

Journal: Manuscript in preparation

The idea to this study was developed by Hawis Madduppa and Marc Kochzius. All sampling was done by Hawis Madduppa together with Agus Nuryanto. The analysis in the laboratory was carried out independently by Hawis Madduppa in the UFT Bremen. The computer analysis was as well carried out by Hawis

Madduppa. The manuscript was written by Hawis Madduppa with revisions and improvements by Marc Kochzius and Janne Timm.

Publication 3

Title: High self-recruitment in anemone fishes revealed by microsatellite DNA parentage analysis

Authors: Hawis H. Madduppa, Janne Timm, and Marc Kochzius

Journal: Manuscript in preparation (PLOS)

The idea to this study was developed by Marc Kochzius and Hawis Madduppa. All sampling was done by Hawis Madduppa together with Agus Nuryanto. The analysis in the laboratory was carried out independently by Hawis Madduppa in the UFT Bremen. The computer analysis was as well carried out by Hawis Madduppa. The manuscript was written by Hawis Madduppa with revisions and improvements by Marc Kochzius and Janne Timm.

Chapter 1

Review of PhD Thesis

**Self-recruitment in anemonefish and
the impact of marine ornamental fishery in
Spermonde Archipelago, Indonesia:
implications for management and conservation**



◀ A colourful *Amphiprion ocellaris* hides under sea anemone *Heteractis magnifica*, and its surrounding coral reef environment at the Spermonde Archipelago

Chapter 1

Review of PhD Thesis:

Self-recruitment in anemonefish and the impact of marine ornamental fishery in Spermonde Archipelago, Indonesia: implications for management and conservation

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1.1 General introduction

The Indonesian Archipelago is situated between two continents (Asia and Australia) and two oceans (Pacific and Indian), the two oceans are connected with the Indonesian Trough Flow (ITF) (Wyrtsky 1961, Fig. 1.1), forming a favourable habitat for many terrestrials and marine organisms (Tomascik et al. 1997). With a coastline stretching over 80,000 kilometres around more than 17,000 islands surrounded by coral reefs with an area of 85,707 km², Indonesia is known as the largest archipelagic nation (Tomascik et al. 1997) and as the centre of marine biodiversity (Myers et al 2000, Roberts et al. 2002, 2000, Allen 2008, Allen and Werner 2002). Various studies have hinted that Indonesia and its adjacent regions to be a biodiversity hotspot for coral reefs (Hughes et al. 2002) and fish (Randall 1998). Indonesia's coral reefs harbour approximately 590 species of corals (Veron 2002) and over 2000 species of reef fish (Allen and Adrim 2003), placing Indonesia at the centre of coral reef diversity in the world, in the so-called Coral Triangle. In addition, in this country five species of sea turtles, 141 species of sea cucumbers, 87 species of sea stars, 84 species of sea urchins, and over 1500 species of molluscs are recorded (Nontji 1993).

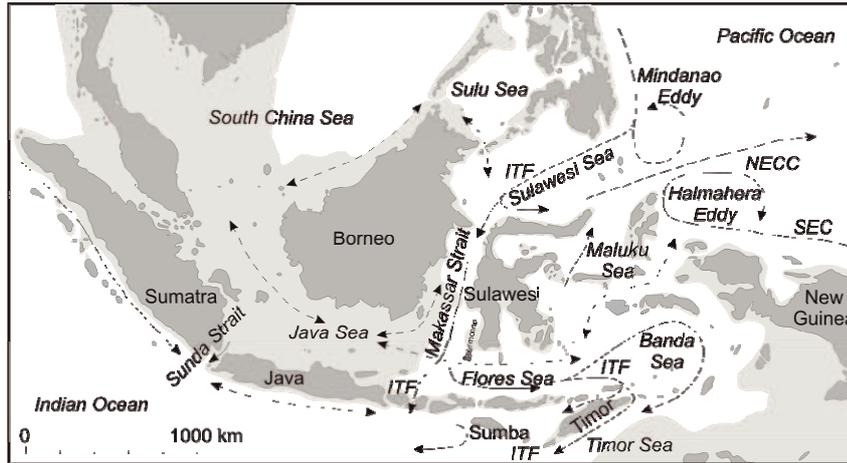


Fig. 1.1: Map of Indonesia showing the direction of Indonesia trough flow from Pacific to Indian Ocean

Indonesia is inhabited by more than 240 million people, with almost 60 million of them living on the coastal area and relying on the coastal and small island ecosystem resources (Burke et al. 2011). While Indonesia is at the hotspot of marine biodiversity and sustains the livelihoods of millions of people, Indonesia's coral reefs are also among the most threatened by anthropogenic factors and other natural threats (Burke et al. 2011). For example, destructive and unsustainable fishing practices (Burke et al. 2011, Edinger et al. 1998, Yoshikawa and Asoh 2004, Pet-Soede et al. 2001, Fox et al. 2003), marine- or land-based pollution and damage (Burke et al. 2011, Edinger et al. 1998), coastal development (Burke et al. 2011), rising ocean temperature (Baker et al. 2004; Spalding and Jarvis 2002), and ocean acidification (Cooley et al. 2009) put pressure on the country's reefs. Therefore, Indonesia urgently needs an appropriate sustainable management system to protect and conserve its biodiversity and its economically importance resources.

The establishment of marine reserves is a well-known strategy for a sustainable use and conservation of coral reef resources (Agardy 1994, Botsford et al. 2003). Marine reserves and fishery closures have proven to increase species diversity of target and nontarget species, as well as large increases in fisheries productivity (Worm et al. 2006). Marine Protected Areas (MPAs) have been proposed and implemented in Indonesia for the

management of its marine fisheries and its marine biodiversity (Alder et al. 1994a, Alder et al. 1994b, Mous et al. 2005). Until now, Indonesia has 248 MPAs; however they cover only 1.94% of its territorial seas (WDPA 2010). In addition, a long-term program (1998-2013) initiated by the Government of Indonesia called COREMAP (Coral Reef Rehabilitation and Management Program), which has among its objectives the protection, rehabilitation, and sustainable use of the Indonesian coral reefs and their associated ecosystems (COREMAP 2010), is currently establishing new protected areas. Unfortunately, some MPAs within the region are not properly working, because newly established MPAs are not well communicated and most local people are not informed about the protection status (Glaser et al. 2010).

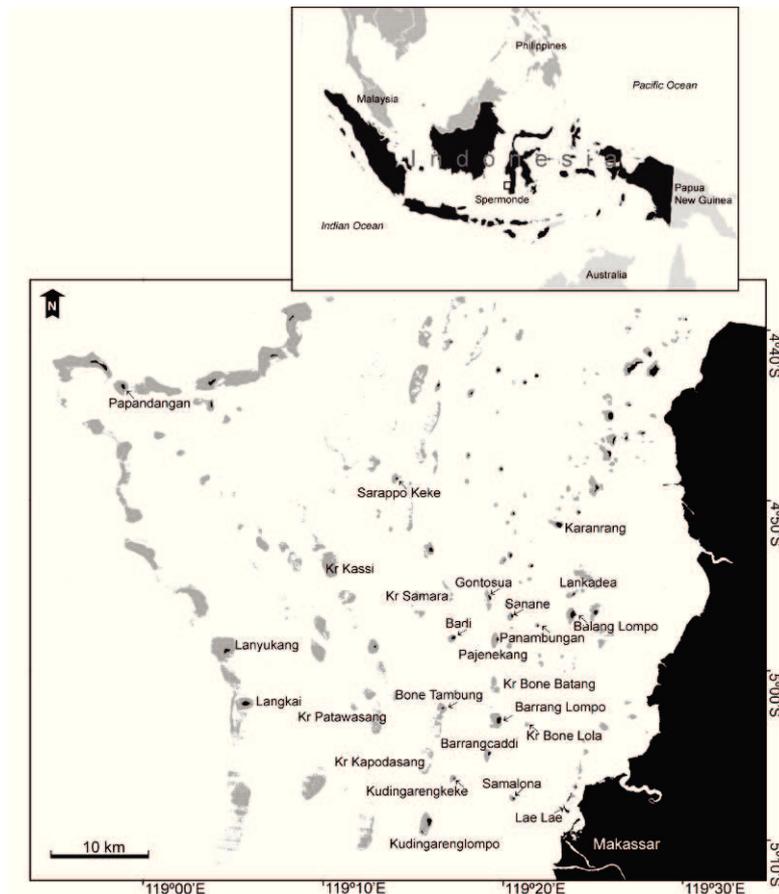


Fig. 1.2: Map of Spermonde Archipelago

The Spermonde Archipelago is located in the Makassar Strait to the southwest of Sulawesi Island, Indonesia and consists of about 150 islands

with a total area of about 400,000 hectares (Tomascik et al. 1997, Fig. 1.2). However, anthropogenic pressures, like many other Indonesian reef system, threaten most of the reefs. Edinger and Risk (2000) reported that bombing and local sewage pollution impacted coral reefs at Barrang Lompo, while in Samalona the impacts have come from anchor damage and pollution from the city. About 12 years ago, the percentage of live coral cover and dead coral in the depth range of 3–10m at Barrang Lompo were 47.21% and 17.83%, respectively, and in Samalona the figures were 44.3% and 15.76%, respectively (Edinger et al. 1998). In addition, coral genera decreased by about 25% from 1985 to 1995 at both islands (Edinger et al. 1998). The Spermonde Archipelago is one of the target sites of COREMAP.

The coral coverage and abundance of marine organisms are bound to decrease further due to high fishing pressure with the use of destructive fishing gear. In addition, marine ornamental trade is an ongoing threat to coral reef organisms in this region due to a lack of appropriate regulation and management applied. An important aspect for the management of living resources is the connectivity of populations and the extent of self-recruitment. These two elements are also important to design and improve marine protected area. The trade and population connectivity are explained in detail below.

1.1.1 Marine ornamental fishery and its impact

The marine aquarium trade is an industry that involves various countries throughout the world. Indonesia and the Philippines are the main exporters, whereas The USA, the European Union, and Japan are the major importers (Wood 2001a; Wabnitz et al. 2003, Fig. 1.3). The volume and value of the marine ornamental trade is enormous. An estimate worth of US \$200–300 million annually are recorded from the trade of fish, coral and other invertebrates (Wabnitz et al. 2003). The proportion of marine ornamental fish imports into the UK increased threefold in terms of total weight from 1977 to 1989 (Andrews 1990). In 1993, Indonesia

received revenue amounting to approximately US \$5.5 million from exports of marine ornamental fish (Wood 2001a).

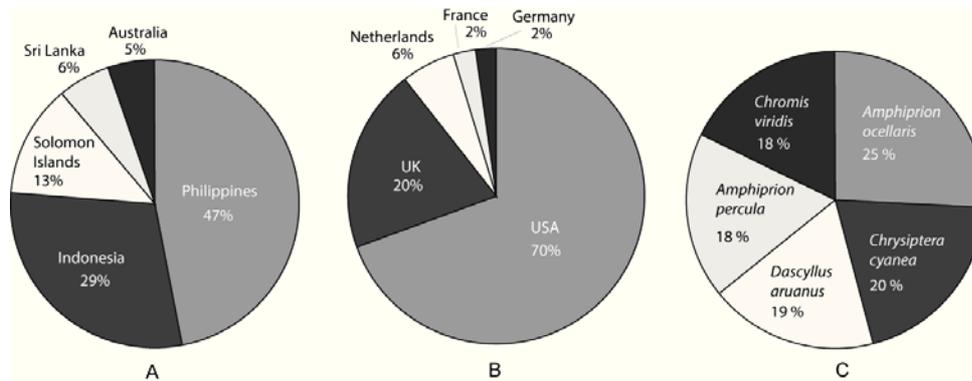


Fig. 1.3: Trade data of marine ornamental fish for the years 1997 to 2002: (A) main exporter countries, (B) main importer countries, and (C) five most traded species of ornamental fish worldwide (source: Wabnitz et al. 2003).

More than one thousand marine fish species were traded globally in the period from 1997 to 2002, of which Pomacentridae comprised about 47% of the total marine ornamental fish traded (Wabnitz et al. 2003). With more than 145,000 specimens collected and traded during this period, the false clown anemonefish *Amphiprion ocellaris* was the number-one species. Anemonefish, such as *A. ocellaris*, are the best example of successfully captive-bred ornamental specimens (Frakes and Hoff, 1982; Madhu et al., 2006; Avella et al., 2007). However, over 90% of marine ornamentals source are still from wild-caught fisheries (Wabnitz et al. 2003) because of limitations in both funds and difficulties in breeding or husbandry, and to be cheaper than rearing fish.

High fishing activities have considerable impacts on the populations of the targeted species. Many authors have discussed the potential impact of the aquarium trade on marine fish species. Jennings et al. (1999) demonstrated that fishing has the effect of slowing the growth rate of larger species leading to later maturity and lower rates of potential population increase. Declining density (Tissot and Hallacher 2003) and diminishing body size (Bianchi et al. 2000) are the most revealing and observable impacts of overfishing on target populations. In Australia, decreased abundance of a species due to collection in a localised region

was observed (Whitehead et al. 1986). In Hawaii, density of aquarium fish species in control site was higher than collection sites (Tissot and Hallacher 2003). In the Philippines, both anemone and anemonefish densities were significantly lower in exploited areas than in protected areas (Shuman et al. 2005). Therefore, many of the coral-reef-associated organisms such as fish are severely overexploited and are being depleted (Musick et al. 2000; Dankel et al. 2008).

Habitat loss and exploitation are driving many marine organisms to extinction (Dulvy et al. 2003). However, many of them would not drive to extinction before their genetic factors affect them. Spielman et al. (2004) found that 77% of the 170 threatened taxa had lower heterozygosity than related non-threatened taxa. Many authors have proven that the fishing activities affect genetic diversity, in addition to decreasing spatial distribution of a population (O'Brien 1994; Heino and Godø 2002; Reusch et al. 2005). The ignorance for the loss genetic diversity might be due to its invisible and long-term effects, compared to the visual impact on the population (Frankham 1995). A study on the population of *Gadus morhua* (e.g., Ottersen et al. 2006) revealed that its size structure, biomass, and diversity declined due to excessive fishing over 60 years.

The genetic consideration is one approach to determine minimum viable population sizes and their area requirements to conserve populations of a particular species (Shaffer 1981). When a population reduced in size, genetic drift enhanced and alleles will eventually be lost (Garza and Williamson 2001). Increased homozygosity and expression of recessive deleterious alleles when they become homozygous (inbreeding depression) are the main result of changes in size and/or composition of the small population (Lande 1988). Declined allelic diversity and expected heterozygosity are observed in bottlenecked populations (England et al. 2003).

Due to the increasing impact on the population of targeted species from the ornamental fishery and other human disturbances, many efforts have been conducted to save these resources. Implementation of marine

protected areas is one example of efforts to manage and to conserve marine living resources.

1.1.2 Self-recruitment and population connectivity

Self-recruitment and the connectivity of populations are a fundamental aspect for the management and conservation of the diversity of living marine resources (Fairweather 1991), management of highly harvested species (Roberts 1997), the population dynamics of marine organisms (Underwood and Fairweather 1989), and improving the design of marine reserves (Almany et al. 2009). Sufficient self-sustaining of marine organisms and their connectivity among populations in marine reserves prevent local extinction because of anthropogenic disturbances such as fishing pressures (Sala et al. 2002).

The number of larvae returning to and settling in their natal population called Self-recruitment, whereas population connectivity is the linking of distinct populations by individual dispersal or migration. Individual organisms or their gametes (Fig. 1.4) mediate the movement of genes between or within populations. However, determining the degree of self-recruitment and the connectivity in marine fishes are challenging issues due to the additional influence of many factors.

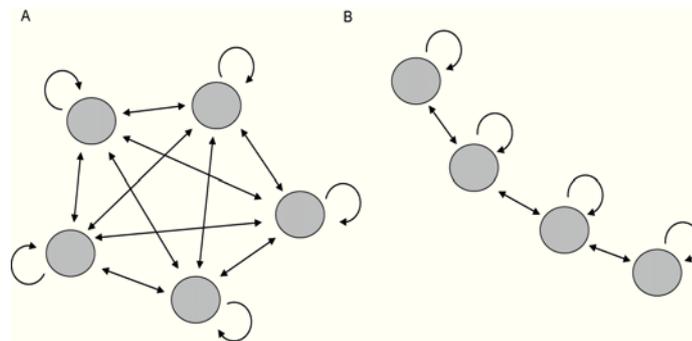


Fig. 1.4: Models of gene flow between populations: (A) island, and (B) stepping-stone model. Arrows shows routes of gene flow. (modified from Beebee and Rowe 2008).

First, the duration of larvae in a pelagic stage, which varies from days to weeks in fish (Wellington and Victor 1989), is related to geographical range size (Lester and Ruttenberg 2005). Anemonefish have a bipartite life cycle like many other marine organisms: they broadcast their eggs and

have a dispersive larval stage that range from days to weeks. The dispersive stage leads them to stay at the pelagic environment for a certain period, after which they settled in an appropriate habitat (Fig. 1.5). This pelagic period affects the dispersal and the potential to connect between populations. This is why marine ecosystem considered as open because it is connected by the dispersal larvae.

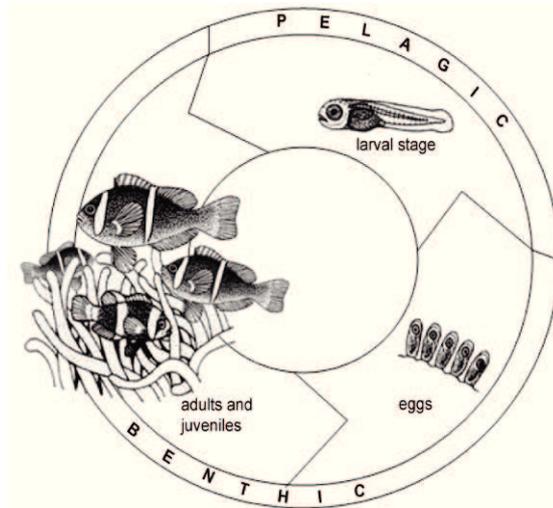


Fig. 1.5: Life cycle in anemonefish: sedentary adults and juveniles, demersal eggs, and dispersing larvae (modified from Fautin and Allen 1994)

Second, although the early stage of larvae is potentially influenced by oceanographic processes (Wilson and Meekan 2001, Sponaugle et al. 2002, Paris and Cowen 2004), the swimming ability and the behaviour of marine fish can determine the distribution pattern and offset the effects of the spread of ocean currents and have the potential to facilitate active self-recruitment in a range of coral reef ecosystem (Fisher and Belwood 2003, Fisher 2005).

Third, larval behaviour such as olfactory reef sensing may be used by the early developmental stages of fish to control their distribution and dispersion (Kingsford et al. 2002), and to orientate to their native habitat (Arvedlund and Nielsen 1996). On the latter, highly specialised associations between anemonefish and their host anemone potentially allow the fish to initiate homing to the origin reef because they imprint themselves to their species-specific host sea anemone using olfactory cues. Olfaction senses are used to carry information on food, reproduction,

social recognition or kinship (Ward et al. 2007), danger, and homing (Munday et al. 2009, Settles 2005, Kingsford 2002, Gerlach et al. 2007). The well-developed sensory and olfactory systems enabled the larva of *A. ocellaris* to detect food on the first day after hatching (Yasir & Qin 2007). Therefore, these species are predicted to have a limited geographic distribution considering their biological characteristics, which in turn increases its vulnerability to high fishing efforts.

Altogether, these factors contribute to the extent of self-recruitment and the connectivity of marine organisms. Many studies have revealed different levels of self-recruitment in marine fishes according to different periods of pelagic larvae (Table 1.1).

Table 1.1: Summary of self-recruitment levels (SR) in organisms with different pelagic larval duration (PLD) and methods used in the different studies.

| Species | PLD (days) | SR (%) | Methods | Reference |
|--------------------------------|------------|--------|---|----------------------------------|
| <i>Tripterygion delaisi</i> | 16–21 | 66 | assignment test and microsatellite loci | Carreras-Carbonell et al. (2007) |
| <i>Pomacentrus amboinensis</i> | 16–19 | 15-60 | otolith marking | Jones et al. (1999) |
| <i>Sebastes melanops</i> | 83–174 | 60-90 | otolith microstructure and microchemistry analysis | Miller and Shanks (2004) |
| <i>Thalassoma bifasciatum</i> | 45 | >70 | larval growth histories and otolith trace-element composition | Swearer et al. (1999) |
| <i>Chaetodon vagabundus</i> | 38 | 60 | Stable barium (Ba) isotopes, otolith marking | Almany et al. (2007) |
| <i>Amphiprion polymnus</i> | 9–12 | 15-30 | tetracycline mass-marking and parentage analysis | Jones et al. (2005) |
| <i>A. percula</i> | 8-12 | 60 | Stable barium (Ba) isotopes, otolith marking | Almany et al. (2007) |
| <i>A. percula</i> | 8-12 | 40 | Parentage analysis and microsatellite | Planes et al. (2009) |
| <i>A. ocellaris</i> | 8-12 | 40-60 | Parentage analysis and microsatellite | This study (Chapter 4) |
| <i>A. perideraion</i> | 18 | 40 | Parentage analysis and microsatellite | This study (Chapter 4) |

1.2 Objectives and studies

The Spermonde Archipelago is one of the main collection sites for the marine ornamental trade in Indonesia. Many coral reef fishes are currently collected for the aquarium trade, of which the clown anemonefish *Amphiprion ocellaris* is the most popular species. The trade is ongoing, but unfortunately the total catch and the potential impact of high exploitation rates are not known. Additionally, no proper management and conservation efforts exist in Indonesia to protect this species. This situation will potentially contribute to the degradation of marine biodiversity. Thus, a proper management, including the implementation and effective enforcement of marine protected areas (MPAs), is needed to avoid overexploitation and depletion of this commercially harvested fish species. An important aspect for the management of living resources is the connectivity of populations and the extent of self-recruitment. Thus, the present thesis follows two main themes: (1) the status and impact of the marine ornamental fishery and (2) self-recruitment of *A. ocellaris*.

1.2.1 The status and impact of the marine ornamental fishery (Chapter 2 and 3)

Exploitation of anemonefish and sea anemones in the Spermonde Archipelago for the marine ornamental industry is not properly recorded, and no quantitative data about the impact are available. The impact of the marine ornamental fishery can be at the population and the genetic level. A significant genetic structure of *Amphiprion ocellaris* and other marine organisms has been found between zones in the Spermonde Archipelago (Timm and Kochzius 2008; Knittweis et al. 2009). Moreover, even though COREMAP constitutes a very significant effort regarding the design of appropriate management plans in this area, yet little effect in the implementation.

Therefore, this study (**Chapter 2**) was performed in order to (1) investigate the amount of collection and trade of *A. ocellaris* and its host anemones in Spermonde Archipelago, (2) investigate the impact of the

ornamental fishery on populations of *A. ocellaris* and its host anemones, and (3) to draw conclusions for management and conservation.

Since effects of fishing on the genetic diversity in the Spermonde Archipelago (Indonesia) are not known so far, a study (**Chapter 3**) was conducted to investigate genetic diversity of the highly traded ornamental fish *Amphiprion ocellaris* in two island populations (Barrang Lompo and Samalona) within the archipelago using eight microsatellite DNA, and to compare them to the fish population density. Knowledge of how the population size relates to the genetic variability in the region would provide an improved understanding of the impact of ornamental fishery, and inform proper management in the region.

1.2.2 Self-recruitment (Chapter 4)

Even though measuring larval dispersal regarded as the greatest challenge facing marine ecologists and managers (Sponaugle et al. 2002, Jones et al. 1999), it is useful for conservation and management of coral reef fishes, and for designing and developing MPAs (Fairweather 1991, Almany et al. 2009). A recent study showed limited connectivity of *Amphiprion ocellaris* populations across Indonesia as well as among shelf areas in the Spermonde Archipelago, predicting high self-recruitment in the mid-shelf area of the archipelago (Timm and Kochzius 2008). Thus, a study (**Chapter 4**) was performed to investigate the degree of self-recruitment, the genetic relatedness, and site fidelity of *A. ocellaris* and *A. perideraion* populations of two small islands in the mid-shelf region (Barrang Lompo and Samalona, which have different levels of exploitation) of the Spermonde Archipelago by polymorphic microsatellite DNA parentage analysis.

Polymorphic microsatellite DNA parentage analysis was facilitating the study of self-recruitment because of the high variability of microsatellite markers allows for fine-scale ecological studies (Selkoe and Toonen 2006). It uses genotype data as the basis for relationship reconstruction based on the maximum likelihood method, where juveniles are assigned to

the most likely parent from a data set of potential parents (Jones and Ardren 2003). This method has been proven to be a powerful tool for investigating self-recruitment in marine fish (Jones et al. 2005; Almany et al. 2007; Planes et al. 2009). A number of studies were able to identify connections among populations by using parentage analysis (Saenz-Agudelo et al. 2009; Saenz-Agudelo et al. 2011). Genetic relatedness of individuals within a group could also give an indication of whether larvae of marine organisms remain in the origin population or disperse to other regions (Buston et al. 2007).

1.3 Summary of materials and methods

1.3.1 Study area

Socio-economic analysis and catch records of the marine ornamental fishery, an ecological survey, and genetic data collection were conducted within the Spermonde Archipelago (Fig. 1.2). The socio-economic marine ornamental fishery survey was conducted on six islands (**Chapter 2**). The ecological survey was conducted at 28 reef sites (**Chapter 2**), which were divided into two categories: high exploited reefs (HE) and low exploited reefs (LE). The genetic data collection was obtained at two islands (Barrang Lompo and Samalona) (**Chapter 3 and 4**).

1.3.2 Study species

The clown anemone fishes *Amphiprion ocellaris* (Cuvier, 1830) belongs to the subgenus *Actinicola*, sub-family Amphiprioninae in the family Pomacentridae. *A. ocellaris* is similar to *A. percula*, which is found in northern Queensland and Melanesia (Fig. 1.6). Their colour and body patterns are similar and nearly identical, but they can be differentiated from their dorsal spines. *A. ocellaris* has 11 (rarely 10) dorsal spines compared to 10 (rarely 9) in *A. percula* (Fautin and Allen 1994). *A. ocellaris* may have a planktonic larval period lasting about 7–11 days like its sibling species *A. percula* (Thresher et al. 1989), and is associated

with three anemones: *Heteractis magnifica*, *Stichodactyla gigantea* and *S. mertensii* (Allen 1991).

The pink anemonefish *Amphiprion perideraion* (Bleeker, 1855) belongs to the subgenus *Phalerebus*, subfamily Amphiprioninae, within the family Pomacentridae (Fig. 1.6). A total of 9 – 10 dorsal spines, 16-17 dorsal soft rays, 2 anal spines, and 12 – 13 anal soft rays (Froese and Pauly 2011) are found in this species. They have an orange color and their fins are transparent. There is one white stripe following the dorsal contour from snout to caudal peduncle (Froese and Pauly 2011) and one white vertical stripe between head and trunk. It has an 18-day pelagic larvae stage (Wellington and Victor 1989) and is associated with four different anemones (*Heteractis magnifica*, *H. crispa*, *Mactodactyls doreenis* and *Stichodactyla gigantea*) (Allen 1991).

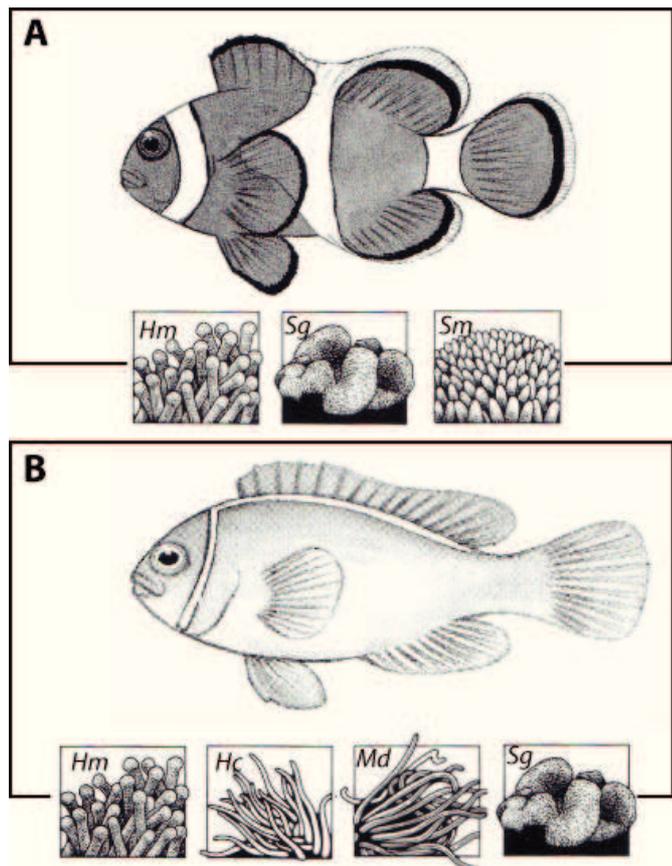


Fig. 1.6: Study species (A) the clown anemonefish *Amphiprion ocellaris* and (B) the pink anemonefish *A. perideraion*, with their potential hosts: Hm = *Heteractis magnifica*, Sg = *Stichodactyla gigantea*, Sm = *Stichodactyla mertensii*, Hc = *Heteractis crispa*, Md= *Macroactyla doreensis* (modified from Fautin and Allen 1994).

1.3.3 Socio-economic data and catch records

Socio-economic survey (September 2005 – March 2006) was conducted at six islands (Sarappo Keke, Barrang Lompo, Karanrang, Lae-Lae, Papandangan, and Balang Lompo) to obtain basic information on the ornamental fishery within the Spermonde Archipelago (*Chapter 2*). Initial information related to socio-economic aspects and marine ornamental activity was obtained from fishermen with a total of 26 respondents who were selected randomly by using purposive sampling and were interviewed using a set of questionnaires. The questionnaire-based interviews combining semi-structured and closed survey questions (Bunce et al. 2000) covered the following: personal data, household members (e.g., name, relation to household head, job), education level, household income, information on the fishery (e.g., fishing ground, fishing gear), and stakeholder identification.

Two surveys were conducted to obtain catch records: September 2005 through March 2006 and September through November 2008 (*Chapter 2*). Since there were no logbooks containing catch composition and volume provided by middlemen and also no actual formal catch records from governmental authorities, information on catches was obtained from interviews with middlemen. The information contained: identity of the middlemen (e.g., name, family status); number, price, and species of ornamental fish and anemone caught per month; fishing ground; fishing gear; and market (wholesaler). Information on the ornamental fish catches was obtained from middlemen on Balang Lompo and Barrang Lompo, while information on sea anemones was obtained on Karanrang and Barrang Lompo.

1.3.4 Ecological survey

Ecological surveys were conducted during two periods: October–November 2008 and May–June 2009 (*Chapter 2*). The first ecological survey was conducted at 28 reef sites within the archipelago to assess the current condition of *Amphiprion ocellaris* populations, together with its

host. These reef sites were divided into two categories: high exploited sites (HE, 14 reef sites) and low exploited sites (LE, 14 reef sites). The last survey was conducted to investigate the impact of the ornamental fishery on *A. ocellaris* populations in terms of body size at two representative islands (Barrang Lompo as HE and Samalona as LE) in more detail.

Free swim surveys were used to census the populations of *Amphiprion ocellaris*. This technique is very useful for covering larger areas (e.g., of more than 100 m in length), and it has been successfully used to census the population of anemone fish in previous studies (e.g., Shuman et al. 2005, Jones et al. 2008). The individual numbers of *A. ocellaris* in one anemone, and the species of anemones in an approximately 5 m-wide area were recorded during free swim surveys. The surveys were carried out for about 1.5–2 hours at a depth of 1–11 m by scuba diving. GPS positions were noted at each survey start and end point. By using surface geographical coordinates, the length of each survey was estimated.

1.3.5 Genetic data collection

Genetic data were collected during October–November 2008 and May–June 2009 (**Chapter 3** and **4**). A total of 364 tissue samples of *Amphiprion ocellaris* and 105 tissue samples of *A. perideraion* were collected using two aquarium nets while scuba diving at fringing reefs of the study sites (Barrang Lompo and Samalona). Tissue samples were taken by a fin clip from the caudal fin and then the fishes were released safely into their host anemones. Each tissue sample was placed into a different tube during this phase of the work. All information regarding the tissue samples (e.g., species, size, date, location, and anemone) was written on underwater slates. Every effort was made to ensure that all individuals in each colony of anemones present at these islands were sampled. The sex of individuals was determined by their body size; the largest fish is the reproductive female, the second-largest fish is the reproductive male, while others remain as non-breeder individuals (Fricke and Fricke 1977; Moyer and

Nakazono 1978). Since this species is monogamous with socially controlled protandrous hermaphroditism (Moyer and Nakazono 1978), this study was able to determine sex quickly during sampling. Tissue samples were preserved in 96% ethanol after the dive and finally stored at 4 °C in the laboratory until extraction.

1.3.6 Laboratory work and data analysis

Laboratory work and data analysis including DNA extraction, PCR amplification and electrophoresis, fragment analysis, allele sizing and correction, and further data analysis were performed according to specific purposes (Fig. 1.7, **Chapter 3** and **4**). Genomic DNA from *Amphiprion ocellaris* and *A. perideraion* were extracted with NucleoSpin Tissue extraction kits from Macherey-Nagel (Germany), following the manufacturer's guidelines. All DNA extracts were analysed by gel electrophoresis to monitor DNA quality prior to PCR (Polymerase chain reaction) amplification of microsatellite loci. GeneRuler™ 1kb DNA Ladder Plus (Applied Biosystems) was used as a reference length.

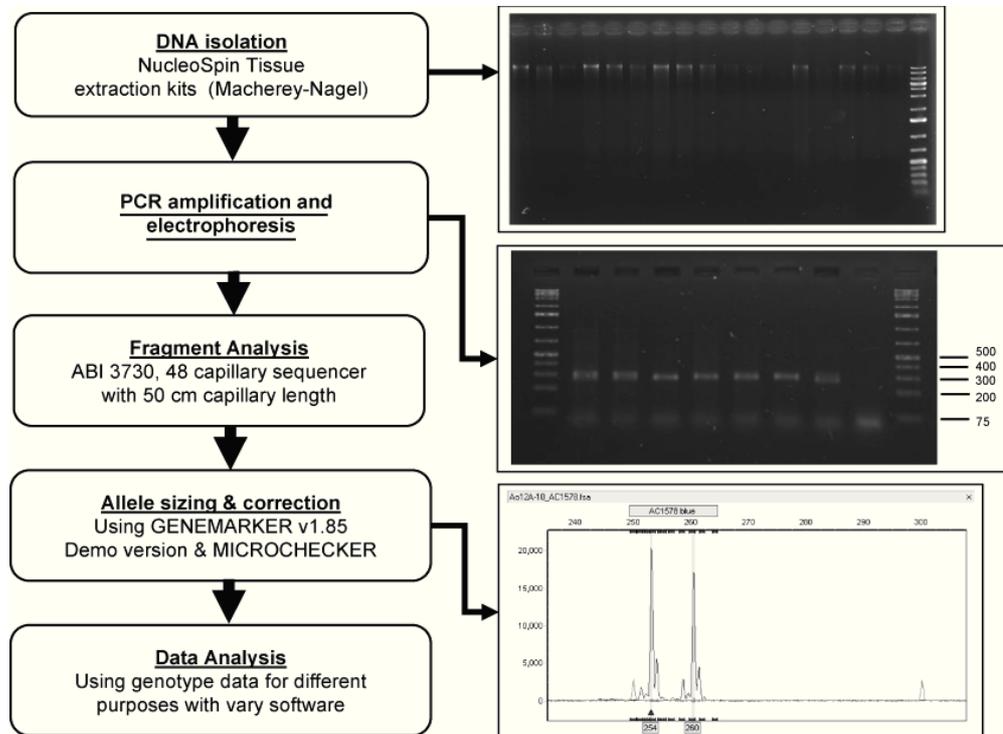


Fig. 1.7: Flow chart of the microsatellite laboratory work, including DNA isolation, microsatellite amplification with the generuler, and allele sizing.

PCRs were carried out in a 25 µl total volume containing 2.5 µl 10x PCR buffer, 3 µl 25 mM MgCl₂, 1 µl 2 mM each dNTP, 1 µl each 10 mM primer forward and reverse, 0.1 µl (5 unit/µl) Taq polymerase (F100L Taq DNA), 1 µl (1-10 ng) genomic DNA. PCRs were performed in a Tprofessional Thermocycler (Biometra) and a Mastercycler ep (Eppendorf) with the following thermo-profile: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s as denaturing step, 50–65 °C for 30 s as annealing step (the optimal annealing temperature varies between primers, see Chapter 4), 72 °C for 1 min for polymerisation, and finally 72 °C for 2 min.

PCR products were diluted in pure water prior to fragment analysis. Then for fragment analysis, 1 µl of diluted PCR product was combined with 8.85 µl HiDi™ formamide and 0.15 µl GENESCAN LIZ-500 size standards (Applied Biosystems). Microsatellite fragments were size-fractionated using an Applied Biosystems Inc. (ABI) 3730 48 capillary sequencer with 50 cm capillary length providing excellent fragment separation and sensitivity. The fragment analysis was conducted in the Department of Biology Genomics Service Unit (GSU), Ludwig Maximilian University, Munich. Allele sizes were determined and corrected with GENEMARKER v1.85 (SoftGenetics GeneMarker). Then the program MICROCHECKER was used to identify and correct irregularities in the data including mistyped allele sizes and typographic errors, scoring errors, and to detect null alleles (Van-Oosterhout et al. 2004).

1.4 Synoptic discussion

1.4.1 Impacts of the marine ornamental fishery (Chapter 2 and 3)

Impact on the population structure (Chapter 2)

The clown anemonefish *A. ocellaris* is the number-one ornamental fish species in the Spermonde Archipelago, and large numbers of it and its host anemones are collected and traded. This species is also the most traded ornamental fish in Bali, the major exporter site in Indonesia (Reksodihardjo-Lilley and Lilley 2007). Even though marine ornamental

resources in the Spermonde Archipelago have been exploited since the beginning of the 1980s (Whitten et al. 2002), only a rather small number of local fishermen are involved in the fishery. However, even though the number of fishermen is comparably small and their activity is spread over the entire archipelago, a fishery targeting almost exclusively on *A. ocellaris* and its host anemones can have a negative impact. In addition, although *A. ocellaris* is a popular fish species among aquarists, the price of this fish is rather low. A low price of anemonefishes is also observed in the Philippines (Shuman et al. 2005). Due to the low price fishermen receive from middlemen, the fishermen spend a huge effort to collect as many anemonefish as possible in order to increase their income.

The trade network persists because of a mutual interdependence between middlemen and fishermen. A similar relationship also exists in India (Sathiadhas and Panikkar 1992) and Malaysia (Merlijn 1989). However, the trade network in the Spermonde Archipelago is more complex due to the mutual interdependence between patron and client (*Punggawa-Sawi*), which is also present in other fishery activities in the region (Schwerdtner Máñez and Ferse 2010).

Poverty and low educational levels add to the problems of the marine ornamental fishery in this area. Even though the monthly expenses per person are above the official Indonesian poverty line of € 17.6 (Rp 211,726) per capita and month (BPS 2010), they are very low. In remote islands, such as Sarappo Keke and Papandangan, where marine ornamental fishery generates a high percentage of the income of marine ornamental fishermen, possibilities for an alternative livelihood are rather limited. Fishermen from islands that are closer to the mainland, e.g. Barrang Lompo, have more opportunities to find an alternative livelihood, because of regular boat transport to Makassar. However, the educational level of marine fishermen family members was generally very low. Most of them only finished elementary school and none of the fishermen's family members had a higher education, which makes it very difficult for them to find a job outside the fishery. Due to the lack of education, most

fishermen do not have much knowledge on the impact of their fishing activities on the environment.

The present study shows that the densities of both *A. ocellaris* and its host anemones were significantly lower in HE reefs than LE reefs. This is probably caused by the high fishing activity for the marine ornamental trade. A comparable study in the Philippines showed a negative impact of marine ornamental fishing on the density and size of both anemones and the anemonefish *A. clarkii* (Shuman et al. 2005). A significant reduction in the density of aquarium fish at collection sites was also observed in Hawaii (Tissot and Hallacher 2003). Aquarium trade affects both the target species (e.g. decreased in species diversity, abundance, and size structure) and the reef community (e.g. reef living space, increased fish parasites as a result of removal of cleaner fish, increased algal coverage as a result of removal of herbivorous fish) in the Great Barrier Reef, Australia (Whitehead et al. 1986). In addition, other phenomena such as bleaching events have a negative impact on the fitness of anemones, due to the loss of its symbiotic zooxanthellae, eventually causing anemone to die. In turn, this negatively affects anemonefishes densities, as observed in the Great Barrier Reef (Jones et al. 2008).

The present study shows that up to eleven individuals of *A. ocellaris* occupied anemones, which was higher than the previously observed 2–6 individuals (Moyer and Nakazono 1978, Fautin 1992). The large group size might interfere with the settlement strategy of post-larval clownfish, which prefer to settle in anemones containing smaller resident groups (Mitchell 2005). This may suggest that some highly exploited reefs had a lack of host anemones, as indicated by the lower density at HE compared to LE. However, at several sites of both exploitation categories, unoccupied anemones were observed. These unoccupied anemones could indicate either that all resident clown anemonefish were collected or that larvae had not settled in this host. Due to their symbiotic relationship, the removal of all fish from one anemone might influence the reproduction, growth rate, and the survival of the sea anemone. It was shown that

anemones hosting *Amphiprion* grow nearly three times faster than anemones without and that the asexual reproduction of anemones was enhanced by their presence (Holbrook and Schmitt 2005).

Extensive harvesting of all size classes at many reef sites in Spermonde Archipelago caused body size reduction. The maximum total length of anemonefish at the island with high exploitation (69 mm) was significantly smaller than at the island with low exploitation (91 mm). However, the maximum total length of anemonefish in both areas was lower than that reported in unexploited populations (110 mm; Allen 1991). This suggests that marine ornamental fishery has a negative impact on the size of anemonefish even at reefs with low exploitation, and overall negative effects of fishery in the area. A similar result was found in *Amphiprion clarkii*, which were larger in protected reefs than non-protected reefs (Shuman et al. 2005). Due to the decrease in body size, fecundity decreases in highly exploited populations (Birkeland and Dayton 2005). Size increase of the male would not necessarily increase the reproductive success, but a larger female can produce more eggs than a smaller one (Fricke and Fricke 1977).

Impact on genetic diversity (Chapter 3)

Gene diversity and expected heterozygosity between Samalona Island and Barrang Lompo Island in the Spermonde Archipelago were found not to differ significantly ($p > 0.05$). Both island populations showed high gene diversity and heterozygosity values (mean H_e and $D > 0.8$). The average expected heterozygosity in *A. ocellaris* for both populations (0.54-0.95) was in a similar range of the previous study on this species by Timm (2008) ranging from 0.54 to 0.92, and many other marine fish species: 0.16-0.91 in *A. percula* (Buston et al. 2007); 0.91 in *Engraulis japonicus* (Yu et al. 2002); 0.77-0.96 in *Gadus morhua* (Bentzen et al. 1996); 0.75-0.85 in *Epinephelus marginatus* (De Innocentiis et al. 2001); 0.69-0.93 in *Dicentrarchus labrax* (García De León et al. 1997), but higher than in some other exploited species, such as *Diplodus sargus* populations ($H_e =$

0.48 ± 0.05) in non-protected areas at the western Mediterranean (Pérez-Ruzafa et al. 2006).

The value of the observed heterozygosity at Samalona was slightly higher than at Barrang Lompo and the heterozygote deficit at Barrang Lompo was higher than at Samalona, but these differences between the two island populations were not significant ($p > 0.05$). Inbreeding in a population could cause decreases in the heterozygosity and increase in the homozygosity. However, at both islands no inbreeding effects were expected based on low inbreeding coefficients (mean 0.067 ± 0.125 at Barrang Lompo and mean 0.042 ± 0.123 at Samalona).

In contrast to the heterozygosity, allelic richness and number of private alleles showed significantly higher values in Samalona than in Barrang Lompo. Some studies have revealed that allelic richness may be more sensitive to a population bottleneck and environmental stress than heterozygosity (e.g., Allendorf 1986; Petit et al. 1998; Leberg 2002; Pini et al. 2011). These results added to the previously observed on the impact of ornamental fishery in the region, because the previous study has revealed the decreased in density and body size in this focal species, due to high collection for marine ornamental trade in the region (**Chapter 2**). Loss of genetic diversity was also found in other exploited species like *Hoplostethus atlanticus* in New Zealand (Smith et al. 1991) and the New Zealand snapper *Pagrus auratus* (Hauser et al. 2002). In the latter, a significant decrease in both heterozygosity and the mean number of alleles over the last 50 years since the onset of exploitation was revealed by seven microsatellite loci. In addition, loss of genetic diversity associated with high mortality was found during the recruitment stage of *Dascyllus aruanus* in Moorea, induced by environmental stress (Pini et al. 2011).

Despite dispersing larvae, *Amphiprion ocellaris* showed significant genetic substructure between the two island populations ($F_{ST} = 0.065 \pm 0.048$, $p = 0.01$). A stronger genetic population structure was also found on a larger scale in the same species as well as in other coral reef organisms in this region (Timm and Kochzius 2008; Knittweis et al. 2009). In

addition, this two island populations revealed high self-recruitment in *A. ocellaris* of about 40-60% (Chapter 4) which indicates most individuals stay in their native population, and possibly structured the population genetically. The value of F_{ST} revealed in the present study is similar with the study on *Acanthochromis polyacanthus* which has no pelagic larvae ($F_{ST} = 0.0603$, Gerlach et al. 2007), and is higher than that found in other marine fish species such as *Amphiprion melanopus* ($F_{ST} = 0.0497$, Doherty et al 1995) and *Ostorhincus doederleini* ($F_{ST} = 0.0189-0.0298$, Gerlach et al. 2007) in the Great Barrier Reef, *Anguilla japonica* ($F_{ST} = 0.002$) in Taiwan (Han et al. 2008), and *Haemulon flavolineatum* ($F_{ST} \approx 0.003$) in the Caribbean (Purcell et al. 2006), as well as in the French Polynesia in eight species of coral reef fishes (F_{ST} –values ranged from 0.0114 to 0.0287, Fauvelot and Planes 2002). Theoretically, levels of genetic differentiation are influenced by various factors; including connectivity, history and size of the populations (Hauser and Carvalho 2008).

The current study has revealed that allelic richness from eight microsatellite loci and fish density in *A. ocellaris* were positively correlated ($p < 0.05$). In other words: the higher the fish density, the more genetic variability; therefore, a decrease in fish density due to exploitation can lead to a reduction of genetic diversity. Similarly, Bazin et al. (2006) observed that the nuclear data (Allozyme) diversity of a given animal species reflects its population size.

1.4.2 Self-recruitment, genetic relatedness, and site fidelity (Chapter 4)

High self-recruitment ranging from 44 to 60.7 % in anemonefishes was revealed in this study. Self-recruitment of *A. ocellaris* (60.7 %) at Samalona was higher than that reported from a previous study on the sibling species *A. percula* (Planes et al. 2009). The high self-recruitment in Samalona is in agreement with evidence of restricted gene flow revealed in the Spermonde Archipelago and across Indonesia (Timm and Kochzius 2008). The high self-recruitment might be triggered by local

currents within the mid-shelf of the Spermonde Archipelago, where the present study sites are located. The level of self-recruitment, found in Samalona, could play an important function in restocking and restoring the archipelago's populations (Lipcius et al. 2008). In contrast, the lower estimated self-recruitment in Barrang Lompo (*Amphiprion ocellaris*: 47.4 %; *A. perideraion*: 43.8 %) is possibly biased by long-term high fishing pressure (Erdmann 1995; Edinger et al. 1998; Chozin 2008). Thus, parent anemonefish are probably removed by ornamental fishermen, deflating the estimate of self-recruitment.

Although many marine fishes have the ability to recognise their relatives in order to avoid inbreeding and competition (Ward and Hart 2003), the analysis of the genetic relatedness of *A. ocellaris* at Barrang Lompo showed that individuals within anemones are closely related, indicating that fish larvae remain in their natal site. This close relatedness between individuals in an anemone may force inbreeding. The close relationship might be due to a low abundance of anemones as a result of their removal for the ornamental fishery, as happening at Barrang Lompo (**Chapter 2**); the pelagic larvae may have find difficulties to avoid staying within their origin or nearby anemones. In addition, anemonefish larvae remain at the first anemone they encounter, without considering the number or identity of residents (Buston 2004). Inbreeding has some consequences, such as deleterious alleles in the first generation because of strictly hierarchical breeding system in anemonefish (Fricke and Fricke 1977, Buston 2004), and reduces the adaptive level of species (Wright 1932). Inbreeding in a population could result in a decrease in the heterozygosity and an increase in the homozygosity, which decreases species fitness and could lead to higher mortality (Charlesworth and Charlesworth 1987). However, no inbreeding effects were expected based on low inbreeding coefficients at Barrang Lompo population (**Chapter 3**).

In contrast, unrelated individuals were identified within the same anemone for *A. ocellaris* at Samalona and *A. perideraion* at Barrang Lompo. These results agree with findings from congeneric individuals of

A. percula (Buston et al. 2007) and *Dascyllus aruanus* (Buston et al. 2009), forming groups consisting of unrelated individuals, which imply that larvae leave their native sites.

This study shows that anemonefish larvae leave their natal anemone, but most of them do not venture far (less than 2 km) from their natal sites. Both islands' populations show that the different sites were connected by dispersal of larvae among the anemones within the island populations. Barrang Lompo and Samalona populations, which are separated by a distance of about 7.5 km, were connected by juveniles' exchange range from 10 to 19 %. Larval dispersal in marine organisms can vary from less than 1 km to 100s km (Swearer et al. 2002; Cowen et al. 2006; Saenz-Agudelo et al. 2009; Salas et al. 2010; Saenz-Agudelo et al. 2011). A study of self-recruitment in *A. polymnus* discovered that even though no individuals settled into the same anemone as their parents, most of them settled close to their origin group (Jones et al. 2005), which is in concordance with the findings in *A. ocellaris* and *A. perideraion* of the current study.

1.4.3 Implications for management and conservation in the Spermonde Archipelago (Chapter 2, 3 and 4)

The results of the present study have implications for the management and conservation on the focal species at the Spermonde Archipelago, Indonesia. Even though the implementation of Marine Protected Areas (MPA) is an important initiative, difficulties in managing and controlling them under local circumstances might be encountered (White 1986; Wood 2001b; Glaser et al. 2010). Many newly established MPAs are not well communicated and most local people are not informed about the protection status (Glaser et al. 2010). Anyway, most of the reef area in Spermonde Archipelago is not protected by an MPA or other management measures. Based on gathered results and information, this thesis has formulated some implications as described below.

The marine ornamental fishery in the Spermonde Archipelago has impacted the population of anemonefish at the population (**Chapter 2**) and genetic level (**Chapter 3**). Indications for over-exploitation of anemonefish and its host anemones may lead to conflicts and social tensions among fishermen as a result of declining and over-fished target populations (Pomeroy et al. 2007). Although the number of fishermen who are engaged in the ornamental fish trade is small (about 3,000 in Spermonde Archipelago), signs of overexploitation are already visible and some populations of *A. ocellaris* and its hosts may collapse (**Chapter 2**). As the current study showed a strong indication that the false clown anemone fish *A. ocellaris* in the two islands (Barrang Lompo and Samalona) was also impacted in the genetic level by the high collection of the wild population, management strategies are required to conserve this targeted species. Kenchington et al. (2003) have proposed several actions in managing loss of genetic diversity in marine species, including: maintaining the number of populations, maintaining the relative size of a population, maintaining a large abundance of individual populations, and minimizing fisheries-induced selection.

The current study suggests that: First, the breeding pair, typically the two largest individual in an anemone, should not be collected. Since this is difficult to control, a maximum catch size of 50 mm should be additionally implemented. Fishermen should only collect the smaller subordinate, because the removal of non-breeders does not affect the survival, growth, and reproductive success of breeding pairs (Buston 2004). Nevertheless, if no breeding pair is present in an anemone, none of the juveniles should be collected to allow them to become reproductive adults.

Second, support the implementation of an MPA network consisting of no-take areas as drawn by COREMAP (DKP 2006); however this should take into account their limited genetic connectivity (Timm and Kochzius 2008) and high amount of self-recruitment (**Chapter 4**) in its design. High levels of self-recruitment imply that the populations are more vulnerable to fishing activity (Thorrold et al. 2001). Information on the proportion of

self-recruitment is important to establish a self-sustaining MPA network (Almany et al. 2007). A high amount of self-recruitment (**Chapter 4**) suggests that single marine protected areas (MPAs) are not suitable as sources for the replenishment of exploited populations. Small MPAs, preferably on every island/reef, should ensure that a part of the population is protected to allow for replenishment by self-recruitment. Such small MPAs are suitable for the archipelago consisting of over 150 islands, most of which are relatively small and many are inhabited. Therefore, a number of small MPAs within the region could ensure the sustainability of the anemone fish populations and prevent them from being depleted.

Fishing prohibition at some zones within MPA could be an effective tool for protecting the genetic biodiversity of target species, which benefits the conservation of allelic richness preserving rare alleles (Pérez-Ruzafa et al. 2006). Since only a small part of the population in the Spermonde Archipelago is engaged in marine ornamental fishery and since it contributes only a minor part to the income (**Chapter 2**), not many people will be affected by restrictions. However, alternative livelihood options have to be investigated for islands where ornamental fishery contributes a high percentage to the livelihood, such as on Sarappo Keke (84 %, **Chapter 2**).

Third, a regular monitoring of the *A. ocellaris* population and its host anemones is necessary to control their status. It is also important that at least middlemen are obliged to keep proper records on the amount and size of collected specimens. This can be enforced and controlled by officer of Ministry of Marine Affairs and Fishery and the Indonesian Statistics Agency, by collecting the catch records directly to middlemen.

Even though the present thesis did not investigate all issues concerning marine ornamental fishery impacts in Spermonde Archipelago, the results can serve as a baseline for the management and conservation of *A. ocellaris* and its host anemones.

1.5 References

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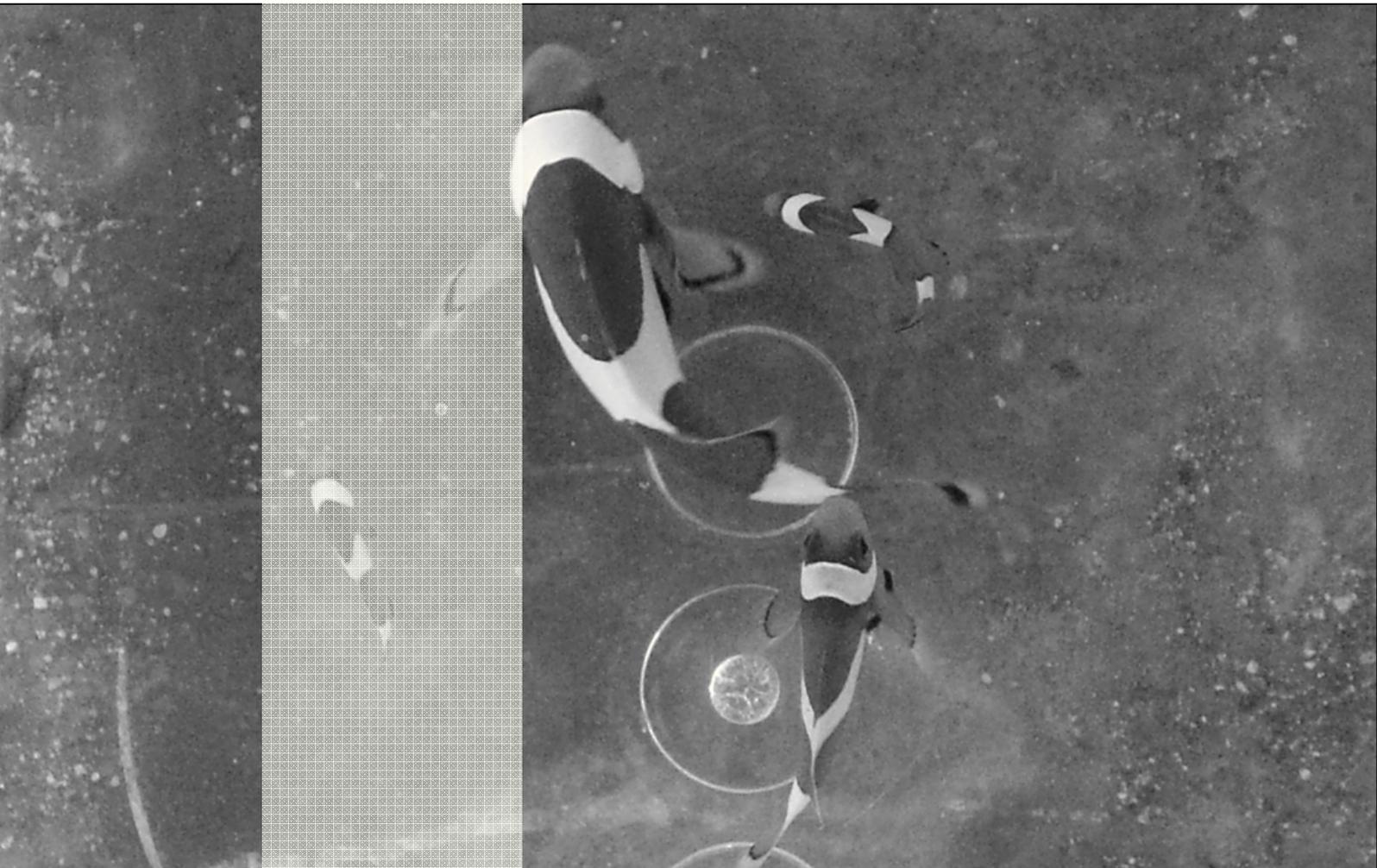
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Chapter 2

Catching Nemo: impact of marine ornamental fishery on the population structure of the clown anemonefish *Amphiprion ocellaris* and its host anemones in the Spermonde Archipelago, Indonesia



◀ A group of *Amphiprion ocellaris* caught near
Barrang Lompo Island, Spermonde
Archipelago

Chapter 2

Catching Nemo: impact of marine ornamental fishery on the population structure of the clown anemonefish *Amphiprion ocellaris* and its host anemones in the Spermonde Archipelago, Indonesia

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2.1 Abstract

Indonesia is one of the major exporters in the international marine ornamental trade and Spermonde Archipelago is one of the main collection sites. The clown anemonefish “Nemo” *Amphiprion ocellaris* is the most popular fish species in the trade. Unfortunately, total catch and the potential impact of high exploitation rates are not known. Additionally, no proper management and conservation efforts exist in Indonesia to protect this species. Therefore, this study aims to (1) investigate the amount of collection and trade of *A. ocellaris* and its host anemones in Spermonde Archipelago, as well as (2) the impact of exploitation on these species. This study revealed that more than 25,000

specimens of *A. ocellaris* (93 % of the total ornamental fish trade) were traded in Balang Lompo over a three-month period in 2005/2006 and more than 10,000 specimens (83 %) were recorded by middlemen on Barrang Lompo in 2008. About 650 anemones were traded by middlemen over a four-month period in 2005 in Karanrang and Barrang Lompo, while during a three-month period in 2008 more than 7,400 anemones were recorded by middlemen on Barrang Lompo. The yearly amount of traded by middlemen on these islands is estimated to about 140,000 specimens *A. ocellaris* and more than 31,000 anemones. Field surveys showed that both *A. ocellaris* and sea anemone densities ($p < 0.01$) were significantly lower at reefs with a high exploitation (HE, 14 sites) than at reefs with a low exploitation (LE, 14 sites). Size of *A. ocellaris* individuals was significantly smaller in Barrang Lompo (HE) than in Samalona (LE) ($p < 0.01$). These findings revealed a considerable negative impact of marine ornamental fishery on the target populations and therefore, have implications for management strategies and conservation.

2.2 Introduction

The marine aquarium trade is an industry which involves various countries throughout the world. A total of 43 supply countries are involved in this industry, of which Indonesia and the Philippines are the main exporters (Wood, 2001). The USA, the European Union, and Japan are the main importers (Wood, 2001a; Wabnitz et al., 2003). An estimated revenue of US\$ 200–300 million annually is recorded from the trade of ornamental fish, corals, and other invertebrates (Wabnitz et al., 2003). Marine ornamental fish imports into the UK increased threefold in terms of total weight from 1977 to 1989 (Andrews, 1990). In 1993, Indonesia received revenue of approximately US \$ 5.5 million from exports of marine ornamental fish (Wood, 2001a). With more than 145,000 specimens traded from 1997 to 2002, the clown anemonefish (*Amphiprion ocellaris*) is the number-one marine ornamental fish species (Wabnitz et al., 2003). Its popularity and demand probably increased due to the animation movie

“Finding Nemo”, featuring the very similar sibling species *A. percula*.

Considering the importance of coral reef fishes for the aquarium trade and in order to reduce overexploitation in the wild, great efforts have been made to rear marine ornamental fish in captivity (Danilowicz and Brown, 1992; Ogawa and Brown, 2001; Johnston et al., 2003). Anemonefish, such as *A. ocellaris*, are the best example of successfully captive-bred ornamental specimens (Frakes and Hoff, 1982; Madhu et al., 2006; Avella et al., 2007). However, most marine ornamentals, in fact over 90 %, are from wild-caught fisheries (Wabnitz et al., 2003), because this appears to be cheaper than rearing fish. Therefore, it seems that production by aquaculture still cannot replace the wild collection of marine ornamental fish (Tlustý, 2002). This suggests that high fishing activities in the wild would have a negative impact on the population level of the targeted species. Fishing can have an effect of slowing down the growth rate of larger species leading to later maturity and lower rates of potential population increase (Jennings et al., 1999). A study on the impact of ornamental fishing on the population structure of anemonefish and its host anemones showed that both anemone and fish densities were significantly lower in exploited areas than in protected areas (Shuman et al., 2005). Besides human pressure, natural phenomena such as bleaching events in coral reefs also negatively influence the density of anemonefish, because the zooxanthellae-bearing host anemones can bleach and die (Jones et al., 2008).

Biological and behavioural characteristics of the clown anemonefish *Amphiprion ocellaris* make it vulnerable to intensive fishing. *A. ocellaris* is widely distributed in the Eastern Indian Ocean and western Pacific, including the Andaman and Nicobar Islands, Thailand, Malaysia, Singapore, Indonesia, north-western Australia, Philippines, as well as northwards to Taiwan and the Ryukyus Islands (Allen, 1991). It lives in close mutualistic association to only three different host anemones (*Heteractis magnifica*, *Stichodactyla gigantea*, and *S. mertensii*), which can be found usually on coastal waters, lagoons and outer reefs at a depth

of 1–31 m (EOL, 2011). Generally, each anemone is inhabited by two to six individuals, where the largest individual in the colony is the reproductive female, the second-ranked individual is the reproductive male, and others remain as non-reproductive individuals (Fricke and Fricke, 1977). In captivity, *A. ocellaris* females spawn 300 to 1 000 eggs per clutch, depending on their body length (Gopakumar et al., 2009). Eggs are laid on the substrate surrounding the anemone (Mitchell, 2003), and after hatching, all anemonefish larvae have a pelagic larval duration (PLD). *A. ocellaris* larvae change from the pelagic to epibenthic stage at an age of 8–10 days in captivity (Gopakumar et al., 2009). *Amphiprion ocellaris* prefers to settle in a smaller group to avoid long queues in order to maximise their likelihood of attaining social dominance (Mitchell, 2005). Settling larva of *A. ocellaris* has a total length of 110 mm in natural populations (Allen, 1991). Reproduction is characterised by protandrous monogamy, i.e. only one breeding pair occurs in a colony occupying a host anemone, and individuals are first males and later change sex to become females. Only the largest dominant male can change sex after the female has died (Fricke and Fricke, 1977; Moyer and Nakazono, 1978). Consequently, all individuals will follow the social-rank rule within one anemone colony by adjusting their size and growth rate (Buston, 2003a). The social structure only changes when one individual dies or is removed from the colony. When the reproductive female is removed, the functional male needs less than one month in a natural population of *A. bicinctus*, two months in captive *A. akallopisos* (Fricke and Fricke, 1977), six months in captive *A. ocellaris* (Madhu et al., 2010), and up to 1.5 years in natural populations of *A. frenatus* (Hattori, 1991) to change sex and to attain female breeding size. When the reproductive male of *A. percula* is removed, estimation of the time required for a non-breeder to mature is about 5 lunar months (Buston, 2004). Therefore, the removing of the two largest individual in an anemone, which are the potential breeding pair, by marine ornamental fishermen can have a profound negative effect on the reproduction of anemonefish populations, especially when sex change

takes 1.5 years.

Because exploitation of anemonefish and its host anemones in the Spermonde Archipelago for the marine ornamental industry is not properly recorded, no quantitative data about the impact are available. Moreover, little effort has been made regarding the design of appropriate management plans in this area. Therefore, this study was performed in order to (1) investigate the amount of collection and trade of *A. ocellaris* and its host anemones in Spermonde Archipelago (2) investigate the impact of ornamental fishery on populations of *A. ocellaris* and its host anemones, and (3) to draw conclusions for management and conservation.

2.3 Methods

2.3.1 Study area

The Spermonde Archipelago is located in the Makassar Strait, southwest of Sulawesi Island, Indonesia (Fig. 2.1) and consists of about 150 islands with a total area of about 400,000 hectares (Tomascik et al., 1997). It is located in the Coral Triangle, which is considered to be the world's center of marine biodiversity (Allen and Werner, 2002; Veron et al., 2009). About 50,000 people live on the islands of Spermonde Archipelago and there are about 6,500 fishing households, which rely on the resources of coral reefs for their livelihood (Pet-Soede et al., 2001). Intense fishing activity for the marine ornamental and live food-fish trade is reported from the area (Erdmann, 1995; Pet-Soede et al., 1999). The use of destructive fishing practices, such as explosives and cyanide is widespread (Pet-Soede and Erdmann, 1998). For instance, coral reefs at Barrang Lompo have been impacted by bombing and local sewage pollution, while reefs in Samalona were impacted by anchor damage and pollution from the city of Makassar (Edinger and Risk, 2000). The percentage of live coral cover and dead coral in the depth range of 3–10 m at Barrang Lompo were about 47 % and 18 %, respectively. In Samalona the figures were 44 % and 16 %, respectively. In addition, diversity (number of coral

genera) decreased by about 25 % from 1985 to 1995 at both islands (Edinger et al., 1998).

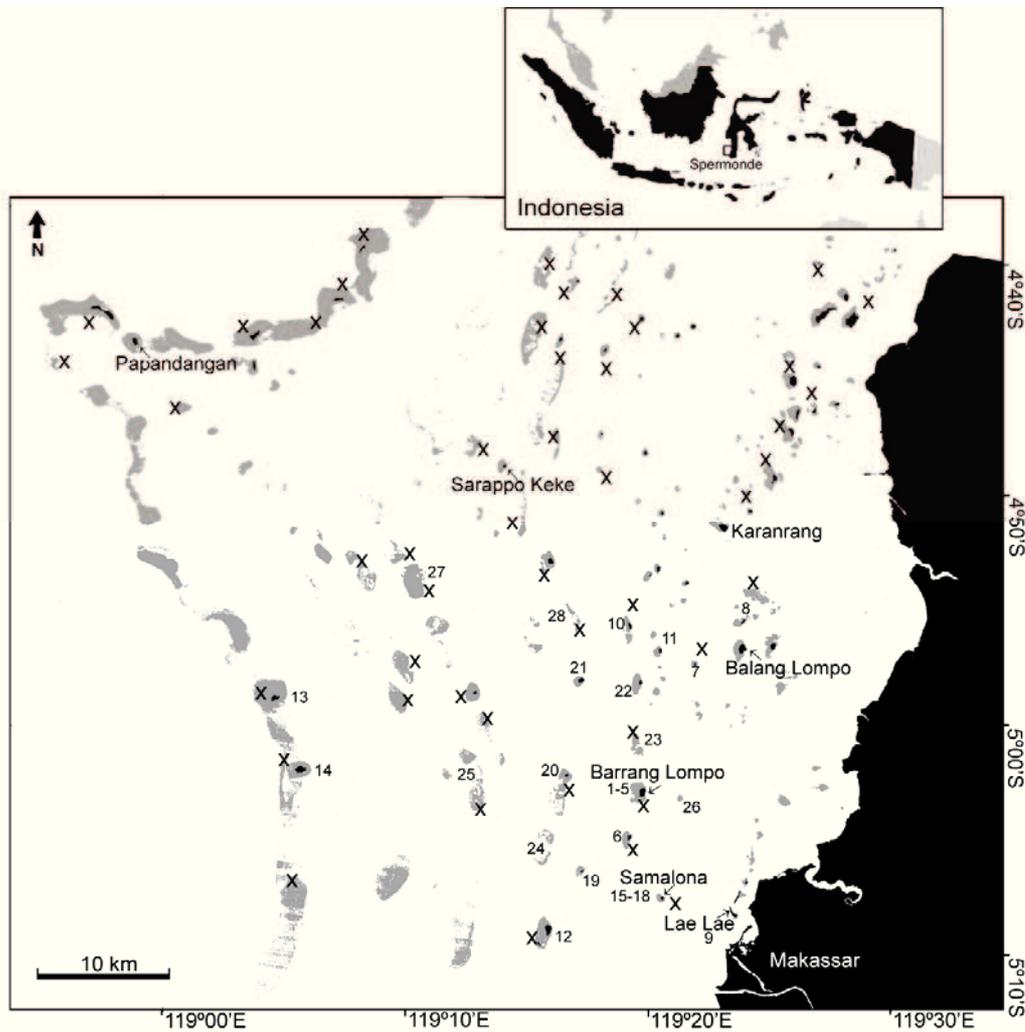


Fig. 2.1: Study sites and fishing grounds (X) in Spermonde Archipelago at the southwestern tip of Sulawesi, Indonesia. Names of islands for the study on the structure and socio-economics of the marine ornamental trade are shown. Numbers indicate studied coral reefs with high exploitation (1-14) and low exploitation (15-28) (see in Table 1).

Table 2.1: Study sites and results of free swim surveys, Spermonde Archipelago (Indonesia). FR = fringing reef, SPR = submerged patch reef, PR = patch reef, * = intensive survey at Barrang Lompo and Samalona Island during May–June 2009, HAB = inhabitant island, FG = fishing ground, NG = not guarded, UHAB = uninhabited island, LG = locally guarded (people who live on the island protect their reefs), § = distance to the closest settlement or inhabited island, MPA = marine protected area, PC = reefs have either been utilised in limited ways or protected due to their physical characteristics (e.g. submerged patch reefs)

| Site | Geographic coordinates | Distance to mainland (km) | Status | Reef type | Length of free swims (m) | Survey area (m ²) | Empty anemones counted | Occupied anemones counted | <i>A. ocellaris</i> counted | Density of <i>A. ocellaris</i> (ind 100m ⁻²) | Density of Anemone (ind 100m ⁻²) | |
|------------------------|-----------------------------|------------------------------|--------|-------------------|--------------------------|-------------------------------|------------------------|---------------------------|-----------------------------|--|--|------|
| High exploitation (HE) | 1. Pulau Barrang Lompo – W* | S 05° 02.541' E 119° 19.355' | 17.25 | HAB, FG | FR | 763 | 3815 | 1 | 12 | 52 | 1.36 | 0.31 |
| | 2. P. Barrang Lompo – SW* | S 05° 03.324' E 119° 19.276' | 17.25 | HAB, FG | FR | 293 | 1465 | 0 | 4 | 26 | 1.77 | 0.27 |
| | 3. P. Barrang Lompo – N* | S 05° 02.507' E 119° 19.571' | 17.25 | HAB, FG | FR | 677 | 3385 | 0 | 7 | 23 | 0.68 | 0.21 |
| | 4. P. Barrang Lompo – S* | S 05° 03.317' E 119° 19.490' | 17.25 | HAB, FG | FR | 395 | 1975 | 0 | 14 | 52 | 2.63 | 0.71 |
| | 5. P. Barrang Lompo – E* | S 05° 03.280' E 119° 19.836' | 17.25 | HAB, FG | FR | 383 | 1915 | 0 | 5 | 30 | 1.57 | 0.26 |
| | 6. P. Barrangcaddi | S 05° 05.095' E 119° 18.934' | 16.44 | HAB, FG | FR | 640 | 3200 | 0 | 5 | 17 | 0.53 | 0.16 |
| | 7. P. Panambungan | S 04° 57.216' E 119° 21.829' | 18.33 | NG, FG | FR | 248 | 1240 | 0 | 1 | 5 | 0.40 | 0.08 |
| | 8. P. Lankadea | S 04° 50.013' E 119° 38.004' | 13.82 | UHAB, NG, FG | FR | 250 | 1250 | 0 | 1 | 3 | 0.24 | 0.08 |
| | 9. P. Lae Lae | S 05° 08.407' E 119° 23.386' | 1.57 | HAB, FG | FR | 52 | 260 | 0 | 0 | 0 | 0.00 | 0.00 |
| | 10. P. Gontosua | S 04° 55.964' E 119° 19.192' | 22.3 | HAB, FG | FR | 116 | 580 | 0 | 1 | 4 | 0.69 | 0.17 |
| | 11. P. Sanane | S 04° 56.812' E 119° 20.209' | 18.87 | HAB | FR | 298 | 1490 | 0 | 2 | 7 | 0.47 | 0.13 |
| | 12. P. Kudingarenglompo | S 05° 08.007' E 119° 15.014' | 15.96 | HAB, FG | FR | 350 | 1750 | 0 | 3 | 8 | 0.46 | 0.17 |
| | 13. P. Lanyukang | S 04° 59.180' E 119° 04.152' | 45.58 | HAB, NG, FG | FR | 141 | 705 | 0 | 0 | 0 | 0.00 | 0.00 |
| | 14. P. Langkai | S 05° 01.677' E 119° 05.228' | 41.27 | HAB, FG | FR | 187 | 935 | 2 | 3 | 7 | 0.75 | 0.32 |
| Low exploitation (LE) | 15. Pulau Samalona – W* | S 05° 07.010' E 119° 20.006' | 7.04 | Touristic, LG, FG | FR | 220 | 1100 | 37 | 15 | 53 | 4.82 | 1.36 |
| | 16. P. Samalona – N* | S 05° 07.009' E 119° 20.007' | 7.04 | Touristic, LG, FG | FR | 560 | 2800 | 0 | 22 | 74 | 2.64 | 0.79 |
| | 17. P. Samalona – E* | S 05° 07.005' E 119° 20.009' | 7.04 | Touristic, LG, FG | FR | 470 | 2350 | 3 | 30 | 113 | 4.81 | 1.28 |
| | 18. P. Samalona – S* | S 05° 07.011' E 119° 20.009' | 7.04 | Touristic, LG, FG | FR | 230 | 1150 | 2 | 14 | 41 | 3.57 | 1.22 |
| | 19. P. Kudingarengkeke | S 05° 06.487' E 119° 17.280' | 20.05 | Private, guarded | FR | 252 | 1260 | 0 | 9 | 27 | 2.14 | 0.71 |
| | 20. P. Bonetambung | S 05° 01.947' E 119° 16.657' | 21.85 | HAB, LG, FG | FR | 447 | 2235 | 0 | 8 | 25 | 1.12 | 0.36 |
| | 21. P. Badi | S 04° 58.358' E 119° 17.057' | 23.03 | MPA ^a | FR | 316 | 1580 | 0 | 3 | 10 | 0.63 | 0.19 |
| | 22. P. Pajenekang | S 04° 58.279' E 119° 19.333' | 18.51 | Guarded | FR | 380 | 1900 | 0 | 0 | 0 | 0.00 | 0.00 |
| | 23. Karang Bonebatang | S 05° 00.911' E 119° 19.376' | 16.61 | PC, ± 17 km §, FG | PR | 277 | 1385 | 0 | 7 | 23 | 1.66 | 0.51 |
| | 24. Kr. Kapodasang | S 05° 05.084' E 119° 15.801' | 22.04 | PC, ± 5 km §, FG | SPR | 40 | 200 | 0 | 18 | 32 | 16.00 | 9.00 |
| | 25. Kr. Patawasang | S 05° 02.015' E 119° 12.004' | 29.9 | PC, ± 5 km § | SPR | 120 | 600 | 0 | 3 | 9 | 1.50 | 0.50 |
| | 26. Kr. Bonelola | S 05° 03.161' E 119° 21.198' | 12.01 | PC, ± 5 km § | SPR | 173 | 865 | 2 | 14 | 43 | 4.97 | 1.62 |
| | 27. Kr. Kassi | S 04° 50.009' E 119° 09.012' | 33.79 | PC, ± 45 km §, FG | PR | 145 | 725 | 0 | 21 | 38 | 5.24 | 2.90 |
| | 28. Kr. Samara | S 04° 55.908' E 119° 16.953' | 27.72 | PC, ± 10 km §, FG | PR | 180 | 900 | 32 | 5 | 17 | 1.89 | 0.56 |

^a(Prayudha and Petrus 2008)

2.3.2 Structure and socio-economics of the ornamental fishery

The survey on the structure and socio-economic aspects of the marine ornamental trade was conducted at six islands (Balang Lompo, Barrang Lompo, Karanrang, Lae Lae, Papandangan, and Sarappo Keke) in Spermonde Archipelago from September 2005 to March 2006 (Fig. 2.1, Table 2.1).

These islands were chosen based on the following considerations: 1) indications of exploitation of coral reefs resources for marine ornamental fisheries, 2) marine ornamental fishermen live on the island, and 3) middlemen are trading on the island. Middlemen are defined as the people who act as an intermediary for a wholesaler or exporter from another area (Wabnitz et al., 2003). Information relating to socio-economic aspects and marine ornamental fishery activity was obtained from marine ornamental fishermen. The 26 respondents were selected randomly by using purposive sampling and were interviewed using a set of questionnaires. These interviews combined semi-structured and closed survey questions (Bunce et al., 2000), covering the following: personal data, educational level of household members, income from marine ornamental fishery, expenses, fishing-related activities (e.g., fishing ground, fishing gear), and stakeholder identification. The following information was solicited in the interviews in order to obtain catch record: species of ornamental fish and anemone caught per month, number of specimens, price per specimen, fishing ground, fishing gear used, and identity of the middlemen.

2.3.3 Marine ornamental catch records of middlemen

Information on the ornamental fish catch record was obtained from a middleman in Balang Lompo, while information on sea anemones was obtained from middlemen in Karanrang and Barrang Lompo (September 2005 – March 2006). Additional information on catch records was obtained from September to November 2008 on Barrang Lompo Island. Since there were no logbooks of middlemen and no records from

governmental authorities containing species composition and amount of animals traded available, information was obtained by interviews with middlemen. Three middlemen are operating on Barrang Lompo Island and all of them were interviewed. Since local names of the animals traded were recorded by the middlemen, a list with the corresponding scientific names was established (Table 2.2), using a fish identification book (Allen et al., 2003) and FishBase (www.fishbase.org). The contribution of taxa in relation to the total amount traded was analysed in order to estimate the relative intensity of harvesting *A. ocellaris* and sea anemones. In addition, prices paid to fishermen by middlemen and to middlemen by wholesalers were recorded to determine how economic incentives may influence resource exploitation.

2.3.4 Field surveys on *Amphiprion ocellaris* and its host anemones

Field surveys were conducted at 28 reef sites within Spermonde Archipelago to assess the abundance of *A. ocellaris* and its host anemones from October to November 2008 (Table 1, Fig. 1). Based on observations and interviews, it was revealed that fishermen tend to exploit rather distant reefs, because the reef area in close vicinity to their home island has already been destroyed or damaged by the use of destructive fishing methods. However, some of them continue to use cyanide to catch fish. There are only fishing restrictions at locations which are privately occupied or owned, locally guarded for tourism purposes, or newly established protected areas. Based on the gathered qualitative information, the 28 reef sites were divided into two groups: high exploitation (HE; n = 14) and low exploitation (LE; n = 14) (Fig.2.1, Table 2.1). High exploitation is defined as close to settlements with high fishing activity, inhabited islands, and not guarded islands. Low exploitation is defined as newly protected, guarded by local islanders, private or tourism islands, reefs which have been utilised to a limited extent or protected naturally by their physical characteristics, such as submerged patch reefs which are sometimes difficult to find by fishermen. Reefs with low exploitation

include locations relatively distant from settlements, since access requires much more fuel for the boats.

Free swim surveys were used to census the populations of *A. ocellaris* in order to anticipate the low abundance of this species and its host anemones in the study area. This technique is very useful for covering larger areas and it has successfully been used to census the population of anemone fish in previous studies (e.g. Shuman et al., 2005; Jones et al., 2008). The abundance of *A. ocellaris* in one anemone and the species of anemones in an approximately 5 m-wide area were recorded during free swim surveys. The surveys were carried out for about 1.5–2 hours at a depth of 1–11 m by scuba diving. Geographic positions were recorded by GPS at each survey start and end point. By using surface geographical coordinates, the length of each survey was estimated.

In order to obtain comprehensive information about the impact of marine ornamental fishery on *A. ocellaris* populations, the total length (TL) for all *A. ocellaris* individuals at five reef sites at Barrang Lompo (HE) and four at Samalona (LE) was measured using a ruler in May and June 2009. All efforts were made to ensure that most *A. ocellaris* individuals within a depth range of 1 to 11 m at the reef slope were recorded.

2.3.5 Analysis of abundance, length data, and group size

Density of *A. ocellaris* and anemones (individuals/ 100 m²) as well as group size (fish/anemone) from underwater counts were standardised to compare different site categories. The density of anemones without resident fish was also considered in order to determine host availability for *A. ocellaris*. The relative size frequencies of *A. ocellaris* were compared between site categories (HE and LE). Poisson regression analysis was used to test the statistical significance of differences in *A. ocellaris* density and anemone density between site categories, and as well group size. To compare the length frequency distributions and group size distributions, Pearson's Chi-Square test was used, which tested for the equality of

proportion in length classes and group sizes between Barrang Lompo and Samalona. All statistical tests were performed using Statistica 7 (StatSoft, 2004).

2.4 Results

2.4.1 Structure and socio-economics of the ornamental fishery

According to the information obtained by interviews with fishermen and middlemen, the first organised marine ornamental fishery in Spermonde Archipelago started in 1988/1989. A marine ornamental export company from Jakarta established a holding facility on the island Satando and marine ornamentals were transported by ship from Makassar to Jakarta. In the 1990s the company moved to Barrang Lompo, because transport to Makassar was easier to facilitate. In 1993/1994 a local wholesaler company from Makassar started to establish collection points on several islands in Spermonde Archipelago and in 1997 a legal permit for the collection of marine ornamentals was issued by the local government. Since then, marine ornamentals are shipped by airplane to Jakarta and Bali, which are the main export airports for marine ornamentals in Indonesia.

A total of 26 respondents (fishermen households) from six islands covered 137 family members. All respondents were male, 18–45 years old, and 12 of the 26 respondents were single. The educational level of marine fishermen family members was low; most of them only finished elementary school (Table 2.2). Only in Sarappo Keke (3.6 %) and Barrang Lompo (3.8 %) some fishermen finished high school. None of the fishermen's family members had a higher education.

The highest annual income from marine ornamental fisheries was attained by fishermen from Sarappo Keke (€ 5,236) and the lowest in Lae Lae (€ 902). The household income analysis at six islands revealed that marine ornamental fishery is not the major source of income, covering 13–43 % of the expenses (Table 2.2). The only exception is ornamental in

fishermen Sarappo Keke, where ornamental fishery covered 84 % of their expenses.

The total population of the Spermonde Archipelago is around 50,000 and more than 12,000 live on the six investigated islands. The median marine ornamental fishermen family size was five persons and it is estimated that about 140 households were involved in ornamental fishery activities on the studied islands. Therefore, the estimated total number of people involved in marine ornamental fishery is about 700 persons, which is about 6 % of the total population on the six investigated islands. Based on an extrapolation to the population of the whole Spermonde Archipelago, about 3000 people depend to some extent on marine ornamental fishery.

The key players in marine ornamental fishery in the Spermonde Archipelago are fishermen (collectors), middlemen (main collectors on the island), and wholesalers (exporters). The ornamental fishermen are people living on the islands. Interviews revealed that each middleman is connected to as many as nine groups of fishermen and one group consists of three to six persons. These groups go fishing according to the demand of the middlemen. The wholesalers come from nearby Makassar as well as Bali and Jakarta, and order specimens of *A. ocellaris* from middlemen. Orders of wholesalers varied in number (500–1000 specimens) and size of anemonefish (10–50 mm). However, almost all of the fish in a single anemone are collected by fishermen and middlemen buy also smaller or bigger specimens. Marine ornamental fishermen reported that they catch fish with a traditional net called *sero*. This kind of fishing net was widely used by many fishermen within Spermonde Archipelago. Fishermen stated that they collect marine ornamentals exclusively in Spermonde Archipelago and 50 fishing grounds could be identified (Figure 2.1). Fishermen from Balang Lompo utilise 20 fishing grounds, from Barrang Lompo 17, from Sarappo Keke and Karanrang eight, from Lae Lae five, and from Papandangan three. They used traditional boats called *lepa-lepa* and *jolloro* (equipped with a compressor), which varied in capacity,

engine power and speed, thus defining the distance they can travel for fishing.

Table 2.2: Summary of socio-economic interviews with respondents from Balang Lompo, Barrang Lompo, Karanrang, Lae Lae, Papandangan, and Sarappo Keke (Spermonde Archipelago, Indonesia; Figure 1), conducted from September 2005 to March 2006.

| | Balang Lompo | Barrang Lompo | Karanrang | Lae Lae | Papandangan | Sarappo Keke |
|---|-------------------------|-----------------------------------|-----------------------------|-------------|-------------|---------------------------|
| Personal data: | | | | | | |
| Number of respondent | 5 | 5 | 5 | 4 | 2 | 5 |
| Age range | 18-23 | 29-35 | 19-35 | 23-28 | 21-30 | 27-45 |
| Sex | Male | Male | Male | Male | Male | Male |
| Year start the ornamental trade activity | 1990 | 1990 | 1994-1995 | 2002 | 2000 | 1999-2000 |
| Marital status (married) | 0 (0 %) | 4 (80 %) | 3 (60 %) | 0 (0 %) | 0 (0 %) | 5 (100 %) |
| Number of family members | 27 | 26 | 25 | 19 | 12 | 28 |
| Household member education level: | | | | | | |
| Pre-school | 0 | 4 (15.4 %) | 3 (12 %) | 0 | 0 | 8 (28.6 %) |
| Drop-out/Illiterate (not finish school) | 10 (37 %) | 3 (11.5 %) | 11 (44 %) | 19 (100 %) | 7 (58.3 %) | 3 (10.7 %) |
| Elementary school (SD) | 17 (63 %) | 18 (69.2 %) | 11 (44 %) | 0 | 5 (41.7 %) | 16 (57.1 %) |
| Junior high school (SMP) | 0 | 0 | 0 | 0 | 0 | 1 (3.6 %) |
| Senior high school (SMA) | 0 | 1 (3.8 %) | 0 | 0 | 0 | 0 |
| Higher education (Diploma) | 0 | 0 | 0 | 0 | 0 | 0 |
| Income and expenses (€): | | | | | | |
| Total annual income from marine ornamental fishery for all interviewed families | 4,364 | 1,964 | 2,347 | 902 | 1,745 | 5,236 |
| Average annual income ± SD | 873 ± 0 | 393 ± 0 | 469 ± 274 | 225 ± 0 | 873 ± 0 | 1047 ± 0 |
| Monthly income per fishermen | 73 | 33 | 39 | 19 | 73 | 87 |
| Total annual expenses | 18 436 | 11 315 | 17 769 | 6 307 | 4 041 | 6 258 |
| Average annual expenses ± SD | 3 687 ± 624 | 2 263 ± 296 | 3 554 ± 219 | 1 557 ± 208 | 2 021 ± 98 | 1 252 ± 203 |
| Monthly expenses per fishermen | 307 | 189 | 296 | 131 | 168 | 104 |
| Percentage expenses covered by income from marine ornamental fishery | 24 % | 17 % | 13 % | 21 % | 43 % | 84 % |
| Fishing related activities: | | | | | | |
| Marine ornamentals collected | fish | anemone, coral, clownfish | anemone, coral | fish | fish | clownfish, anemone, coral |
| Fishing gear | sero, sodo ¹ | sero, hammer, compressor, crowbar | compressor, hammer, crowbar | sero (net) | sero (net) | sero, sodo ¹ |

Exchange rate (€ 1 ~ Rp 11,000 September 2005)

Table 2.3: Trade record data for ornamental fish catch recorded in Balang Lompo from November 2005 to January 2006 and ornamental anemones in Karanrang and Barrang Lompo from September to December 2005 from middlemen. Additional catch record data for September to November 2008 from three marine aquarium trade middlemen in Barrang Lompo, Spermonde Archipelago, Indonesia (€ = Euro, Unit price = price paid to fishermen by middleman on the island for individual fish (F) and price paid to middleman by wholesalers from other areas (mainland) for individual fish (M), Total value = total value received by fishermen from middleman on the island for respective fish (F) and total value received by middleman from wholesalers from other areas (mainland) for respective fish (M), * = fishermen made a differentiation between sea anemones based on their colour)

| Species | English name | Local name | Number | | Unit price (F) | | Unit price (M) | | Total value (F) | | Total value (M) | |
|-------------------------------|--------------------------|------------------------|--------------|-------|----------------|------|----------------|------|-------------------|-----------------|-------------------|-----------------|
| | | | n | % | Rp. | € | Rp. | € | Rp. | € | Rp. | € |
| Periode 2005/2006 | | | | | | | | | | | | |
| Ornamental fish | | | | | | | | | | | | |
| <i>Amphiprion ocellaris</i> | False clown anemonefish | Klonpis/ lar' -jappe' | 25 515 | 91.89 | 500 | 0.05 | 1 500 | 0.14 | 12 757 500 | 1 159.77 | 38,272,500 | 3,479.32 |
| <i>A. sandaracinos</i> | Golden anemonefish | Pelet akal/geger putih | 187 | 0.67 | 500 | 0.05 | 1 000 | 0.09 | 93 500 | 8.50 | 187,000 | 17.00 |
| <i>A. frenatus</i> | Oneband anemonefish | Tompel | 70 | 0.25 | 500 | 0.05 | 1 000 | 0.09 | 35 000 | 3.18 | 70,000 | 6.36 |
| <i>A. clarkii</i> | Brown anemonefish | Giro pasir | 55 | 0.20 | 1 000 | 0.09 | 1 500 | 0.14 | 55 000 | 5.00 | 82,500 | 7.50 |
| <i>Premnas biaculeatus</i> | Spinecheek anemonefish | Balong | 72 | 0.98 | 1 000 | 0.09 | 1 500 | 0.14 | 272 000 | 24.73 | 408,000 | 37.09 |
| <i>Chrysiptera cyanea</i> | Cornflower sargeantmajor | Beto-beto | 1 400 | 5.04 | 500 | 0.05 | 600 | 0.05 | 700 000 | 63.64 | 840,000 | 76.36 |
| <i>Dascyllus</i> sp. | Damselfish | Ikan zebra/dakocan kb | 10 | 0.40 | 500 | 0.05 | 600 | 0.05 | 55 000 | 5.00 | 66,000 | 6.00 |
| <i>Zanclus cornutus</i> | Moorish idol | Moris | 50 | 0.18 | 2 000 | 0.18 | 2 500 | 0.23 | 100 000 | 9.09 | 125,000 | 11.36 |
| <i>Zebrasoma scopas</i> | Twotone tang | Burung laut | 50 | 0.18 | 2 000 | 0.18 | 2 500 | 0.23 | 100 000 | 9.09 | 125,000 | 11.36 |
| <i>Halichoeres</i> sp. | Wrasse | Pello/Laccukang | 9 | 0.10 | 700 | 0.06 | 1 000 | 0.09 | 20 300 | 1.85 | 29,000 | 2.64 |
| <i>Chaetodon speculum</i> | Mirror butterflyfish | Kepekepe bulan | 15 | 0.05 | 1 000 | 0.09 | 1 500 | 0.14 | 15 000 | 1.36 | 22,500 | 2.05 |
| <i>Neoglyphidodon oxyodon</i> | Javanese damsel | Blueband | 7 | 0.03 | 1 000 | 0.09 | 1 500 | 0.14 | 7 000 | 0.64 | 10,500 | 0.95 |
| <i>Hemigymnus melapterus</i> | Blackeye thicklip | Tikusan | 5 | 0.02 | 1 000 | 0.09 | 1 500 | 0.14 | 5 000 | 0.45 | 7,500 | 0.68 |
| <i>Pterois</i> sp. | Lionfish | Scorpio/ikan lepu | 2 | 0.01 | 1 500 | 0.14 | 2 000 | 0.18 | 3 000 | 0.27 | 4,000 | 0.36 |
| Total | | | 7 767 | | | | | | 14 218 300 | 1 292.57 | 40,249,500 | 3,659.05 |
| Sea anemone*: | | | | | | | | | | | | |
| <i>Entacmaea quadricolor</i> | Bubble-tip anemone | Anemon jagung biasa | 37 | 4.73 | 1 500 | 0.14 | 2 500 | 0.23 | 55 500 | 5.05 | 92,500 | 8.41 |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Anemon Violet | 63 | 8.06 | 3 000 | 0.27 | 4 000 | 0.36 | 189 000 | 17.18 | 252,000 | 22.91 |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Pantat merah biasa | 541 | 69.18 | 3 000 | 0.27 | 5 000 | 0.45 | 1 623 000 | 147.55 | 2,705,000 | 245.91 |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Pantat Ijo | 58 | 7.42 | 5 000 | 0.45 | 7 000 | 0.64 | 290 000 | 26.36 | 406,000 | 36.91 |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Pantat pendek | 12 | 1.53 | 1 500 | 0.14 | 2 500 | 0.23 | 18 000 | 1.64 | 30,000 | 2.73 |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Pantat putih | 44 | 5.63 | 1 000 | 0.09 | 3 000 | 0.27 | 44 000 | 4.00 | 132,000 | 12.00 |
| <i>Stichodactyla</i> sp. | Flat/carpet anemone | Karpet ijo | 16 | 2.05 | 5 000 | 0.45 | 20 000 | 1.82 | 80 000 | 7.27 | 320,000 | 29.09 |
| <i>Stichodactyla</i> sp. | Flat/carpet anemone | Karpet merah | 4 | 0.51 | 50 000 | 4.55 | 100 000 | 9.09 | 200 000 | 18.18 | 400,000 | 36.36 |
| <i>Stichodactyla</i> sp. | Flat/carpet anemone | Karpet biru | 7 | 0.90 | 20 000 | 1.82 | 25 000 | 2.27 | 140 000 | 12.73 | 175,000 | 15.91 |
| Total | | | 782 | | | | | | 2 639 500 | 239.95 | 4,512,500 | 410.23 |

| Periode 2008 | | | | | | | | | | | | |
|--------------------------------|----------------------------|------------------------|---------------|-------|---------|-------|---------|-------|-------------------|-----------------|-------------------|-----------------|
| Ornamental fish | | | | | | | | | | | | |
| <i>Amphiprion ocellaris</i> | False clown anemonefish | klonpis/ lar'-jappe' | 10 300 | 83.14 | 800 | 0.07 | 2 000 | 0.17 | 8 240 000 | 686.67 | 20,600,000 | 1,716.67 |
| <i>A. sandaracinos</i> | Golden anemonefish | Pelet akal/geger putih | 170 | 1.37 | 500 | 0.04 | 1 200 | 0.10 | 85 000 | 7.08 | 204,000 | 17.00 |
| <i>A. frenatus</i> | Oneband anemonefish | Tompel | 800 | 6.46 | 500 | 0.04 | 1 200 | 0.10 | 400 000 | 33.33 | 960,000 | 80.00 |
| <i>Premnas biaculeatus</i> | Spinecheek anemonefish | Balong | 80 | 0.65 | 1 000 | 0.08 | 1 500 | 0.13 | 80 000 | 6.67 | 120,000 | 10.00 |
| <i>Chaetodon</i> sp. | Butterflyfish | Kepe-kepe | 90 | 0.73 | 1 000 | 0.08 | 1 500 | 0.13 | 90 000 | 7.50 | 135,000 | 11.25 |
| <i>Chrysiptera cyanea</i> | Cornflower sergeantmajor | Beto-beto | 350 | 2.83 | 500 | 0.04 | 600 | 0.05 | 175 000 | 14.58 | 210,000 | 17.50 |
| <i>Chrysiptera brownriggii</i> | Surge demoiselle | Betok asli | 200 | 1.61 | 1 000 | 0.08 | 1 500 | 0.13 | 200 000 | 16.67 | 300,000 | 25.00 |
| <i>Dascyllus aruanus</i> | Black-and-white damselfish | Ikan zebra/dakocan kb | 300 | 2.42 | 500 | 0.04 | 600 | 0.05 | 150 000 | 12.50 | 180,000 | 15.00 |
| <i>Halichoeres scapularis</i> | Zigzag wrasse | Pello | 60 | 0.48 | 800 | 0.07 | 1 000 | 0.08 | 48 000 | 4.00 | 60,000 | 5.00 |
| <i>Labroides dimidiatus</i> | Bluestreak cleaner wrasse | Ikan dokter | 9 | 0.07 | 2 000 | 0.17 | 2 500 | 0.21 | 18 000 | 1.50 | 22,500 | 1.88 |
| <i>Pterois</i> sp. | Lionfish | Ikan Lepu | 18 | 0.15 | 1 500 | 0.13 | 2 000 | 0.17 | 27 000 | 2.25 | 36,000 | 3.00 |
| <i>Pygoplites diacanthus</i> | Bluebanded angelfish | Enjel-enjel | 12 | 0.10 | 7 000 | 0.58 | 10 000 | 0.83 | 84 000 | 7.00 | 120,000 | 10.00 |
| Total | | | 12 389 | | | | | | 9 597 000 | 799.75 | 22,947,500 | 1,912.29 |
| Sea anemone*: | | | | | | | | | | | | |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Anemone putih | 5 000 | 67.36 | 8 000 | 0.67 | 15 000 | 1.25 | 40 000 000 | 3,333.33 | 75,000,000 | 6,250.00 |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Anemone coklat | 1 350 | 18.19 | 8 000 | 0.67 | 10 000 | 0.83 | 10 800 000 | 900.00 | 13,500,000 | 1,125.00 |
| <i>Heteractis</i> sp. | Magnificent sea anemone | Anemone susu | 1 050 | 14.15 | 10 000 | 0.83 | 10 000 | 0.83 | 10 500 000 | 875.00 | 10,500,000 | 875.00 |
| <i>Stichodactyla</i> sp. | Flat/carpet anemone | Karpet biru | 22 | 0.30 | 20 000 | 1.67 | 30 000 | 2.50 | 440 000 | 36.67 | 660,000 | 55.00 |
| <i>Stichodactyla</i> sp. | Flat/carpet anemone | Karpet merah | 1 | 0.01 | 150 000 | 12.50 | 180 000 | 15.00 | 150 000 | 12.50 | 180,000 | 15.00 |
| Total | | | 7 423 | | | | | | 61 890 000 | 5,157.50 | 99,840,000 | 8,320.00 |

Exchange rate (€ 1 ~ Rp 11,000 September 2005 and ~Rp 12,000 November 2008)

2.4.2 Marine ornamental catch records of middlemen

A total of 27,267 fish (14 taxa) and 659 anemones (3 taxa) were recorded from September 2005 to March 2006 in Balang Lompo, Karanrang, and Barrang Lompo. The most abundant fish species was *A. ocellaris* (92 %), and the most abundant anemone was *Heteractis* sp. (92 %) (Table 2.3). More than 25 000 specimens of *A. ocellaris* were collected, with a value of more than € 1,800 (Indonesian Rupiah, Rp 20,400,000) for the fishermen and € 4,600 (Rp 50,000,000) for the middlemen. Fishermen received from middlemen about € 0.05 per *A. ocellaris* specimen, and middlemen from wholesalers about € 0.14. The other four anemonefish (*A. larkia*, *A. frenatus*, *A. sandaracinos*, and *Premnas biaculeatus*) were collected in a small numbers (about 500 specimens in total), with a value of about € 40 (Rp 450,000) for the fishermen and € 60 (Rp 700,000) for the middlemen. Fishermen received from middlemen € 0.05 per specimen of *A. sandaracinos* and *A. frenatus*, and middlemen from wholesalers € 0.09. For *A. larkia* and *Premnas biaculeatus*, fishermen received from middlemen € 0.09 per specimen, and middlemen from wholesalers € 0.14. About 5 % of the total fish catch was *Chrysiptera cyanea*, with a value of about € 60 (Rp 700,000) for the fishermen and € 76 (Rp 840,000) for the middlemen. About 1 % of the total fish catch were other ornamental fish species (*Chaetodon speculum*, *Dascyllus* sp., *Halichoeres* sp., *Hemigymnus melapterus*, *Neoglyphidodon oxyodon*, *Pterois* sp., *Zanclus cornutus*, and *Zebrasoma picen*), with a revenue of about € 25 (Rp 300,000) for the fishermen and € 35 (Rp 380,000) for the middlemen. Fishermen received € 0.05 to 0.18 per specimen and the wholesalers paid € 0.05 to 0.23 per specimen to middlemen.

Catch record data from September to December 2005 from middlemen in Karanrang and Barrang Lompo showed that more than 700 specimens of three anemone species (*Heteractis* sp.: 92 %, *Entacmaea quadricolor*: 5 %, and *Stichodactyla* sp.: 3 %) were collected (Table 2.2). The value for fishermen was about € 240 (Rp 2,600,000) and for middlemen € 400 (Rp

4,500,000). The fishermen received prices from € 0.09-0.45 for *Heteractis* sp., € 0.14 for *Entacmaea quadricolor* and € 0.45-4.55 for *Stichodactyla* sp. Per specimen from middlemen. Middlemen received € 0.23-0.64 for *Heteractis* spp. € 0.23 for *Entacmaea quadricolor* and € 1.82-9.09 for *Stichodactyla* spp. Per specimen from wholesalers.

Catch record data of three middlemen from Barrang Lompo (September to November 2008) showed that more than 12,000 individual ornamental fish belonging to 12 species were traded (Table 2.2). The most abundant species was *A. ocellaris*, representing more than 10,000 specimens (83 %) and a value of more than € 600 (Rp 8,000,000) for the fishermen and € 1,700 (Rp 20,000,000) for middlemen. Fishermen received from middlemen € 0.07 per *A. ocellaris* specimen, and middlemen from wholesalers € 0.17. The revenue of other species of the genus *Amphiprion* was lower, fishermen received from middlemen € 0.04 and middlemen from wholesalers € 0.10 per specimen. The rather rare anemonefish *Premnas biaculeatus* was only collected in low numbers, with revenue of € 0.08 for fishermen and € 0.13 for middlemen per specimen. About 8 % of the total fish catch were other coral reef fish species (*Chaetodon* sp., *Chrysiptera cyanea*, *C. leucopama*, *Dascyllus aruanus*, *Halichoeres scapularis*, *Labroides dimidiatus*, *Pterois* sp., and *Pygoplites diacanthus*), with a revenue of about € 60 (Rp 700,000) for the fishermen and € 80 (Rp 10,000,000) for the middlemen. Fishermen received € 0.04 to 0.58 per specimen and the wholesalers paid € 0.05 to 0.83 per specimen to middlemen.

Catch record data of three middlemen from Barrang Lompo (September to November 2008) showed that more than 7,400 sea anemones of the genera *Heteractis* (> 99 %) and *Stichodactyla* (< 1 %) were collected (Table 2.2). The value for fishermen was € 5,100 (Rp 6,100,000) and for middlemen € 6,600 (Rp 80,000,000). The fishermen received prices from € 0.8–1.1 for *Heteractis* spp. And € 2–15.8 (*Stichodactyla* sp.) per specimen from middlemen. Middlemen received € 1–1.6 for *Heteractis* sp. And € 3–19 for *Stichodactyla* sp. from wholesalers.

2.4.3 Field surveys on *Amphiprion ocellaris* and its host anemones

A total of 739 *A. ocellaris* and 227 sea anemones were recorded during the free swim surveys (Table 2.1). Three different host anemones were utilised by *A. ocellaris* in different proportions: *Heteractis magnifica* (94 %), *Stichodactyla mertensii* (4 %), and *S. picentre* (2 %). Each anemone was occupied by up to eleven individuals of *A. ocellaris* at both categories. The number of anemonefish per anemone were not significantly different between LE and HE reefs (Pearson's chi-square test: $X^2 = 4.84$, d.f. = 8, $P = 0.77$; Fig.2.2). *Amphiprion ocellaris* and its host anemone were not found at three HE sites (Lae Lae, Pajenekang, and Lanyukang). In addition, a total of 79 empty anemone individuals were observed at two HE sites (Barrang Lompo and Langkai) and at three LE sites (Samalona, Bonelola, and Karangkassi) (Table 2.1).

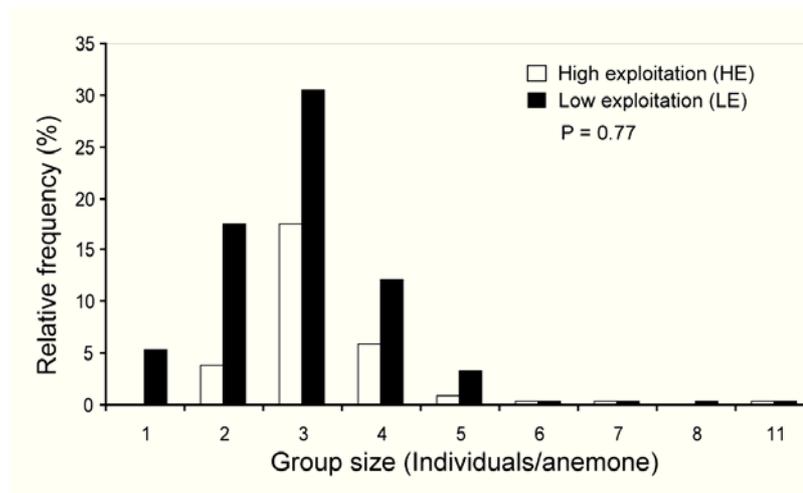


Fig. 2.2: Group size distribution of *Amphiprion ocellaris* in reefs with high (HE, n = 70) and low exploitation (LE, n = 169), Spermonde Archipelago (Indonesia). There is no statistical difference between site categories (Pearson's chi-square test: $X^2 = 4.84$, d.f. = 8, $P = 0.77$).

The density (mean, number of individuals/100 m² ± SE) of *A. ocellaris* during the survey was 0.83 ± 0.20 (HE) and 3.64 ± 1.06 (LE), with a maximum density of 16 individuals (LE). The density of anemones was 0.21 ± 0.05 (LE) and 1.50 ± 0.61 (HE), with a maximum density of 9 individuals (LE). The densities of *A. ocellaris* (Poisson regression: Wald Stat. = 116, d.f. = 1, $p < 0.01$) and its host anemones (Poisson regression:

Wald Stat. = 57.29, d.f. = 1, $p < 0.01$) were significantly lower at HE than at LE. The group size (fish/anemone) was 3.4 ± 0.5 (HE) and 2.8 ± 0.3 (LE), and was not significantly differed between HE and LE (Poisson regression: Wald Stat.= 0.26, d.f. = 1, $p = 0.611$) (Fig.2.3).

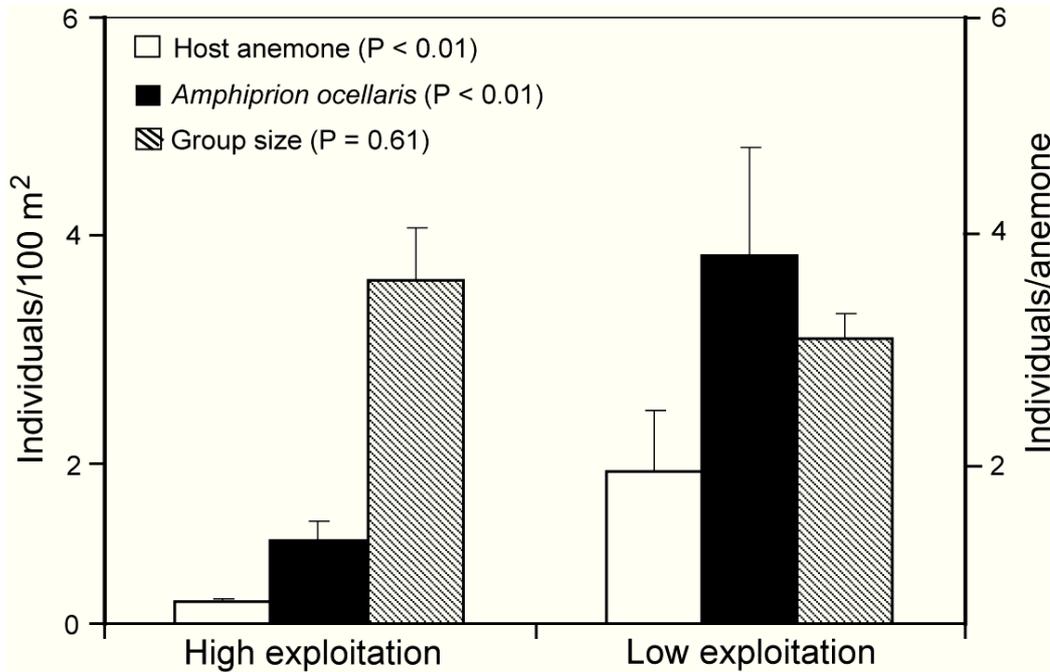


Fig. 2.3: Density (mean \pm SE, individuals/ 100 m²) of *Amphiprion ocellaris* (n = 739) and its host anemones (n = 227) at reefs with high (HE; n = 14) and low exploitation (LE; n = 14), and group size (mean \pm SE individuals/anemone), Spermonde Archipelago (Indonesia). Differences between HE and LE are significant in *A. ocellaris* (Poisson regression: Wald Stat. = 116, d.f. = 1, $P < 0.01$) and its host anemones (Poisson regression: Wald Stat. = 57.29, d.f. = 1, $p < 0.01$), while group size does not differed significantly between HE and LE (Poisson regression: Wald Stat. = 0.26, d.f. = 1, $p = 0.611$).

The length frequency distribution of *A. ocellaris* indicated that specimens in Samalona (LE; TL = 51.43 ± 1.02 mm; n = 183) were significantly larger than in Barrang Lompo (HE; TL = 36.61 ± 1.06 mm; n = 281) (Pearson's chi-square test: $\chi^2 = 84.27$, d.f. = 9, $P < 0.01$; Fig. 2.4). The maximum TL in Samalona was 91 mm, whereas in Barrang Lompo it was 69 mm.

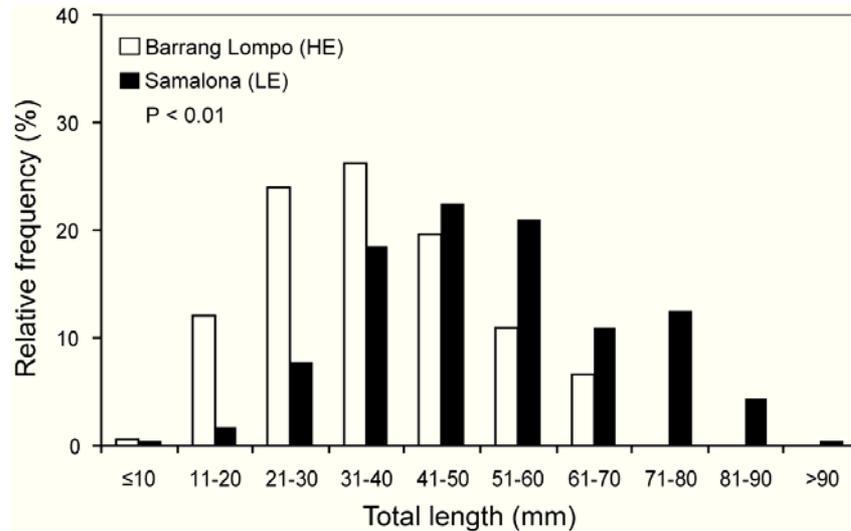


Fig. 2.4: Length frequency distribution of *Amphiprion ocellaris* in Barrang Lompo ($n = 183$; reef with high exploitation, HE) and Samalona ($n = 281$; reef with low exploitation; LE), Spermonde Archipelago (Indonesia). The mean total length is significantly larger at HE than at LE (Pearson's chi-square test: $\chi^2 = 84.27$, d.f. = 9, $P < 0.01$).

2.5 Discussion

2.5.1 Marine ornamental fishery

The clown anemonefish *A. ocellaris* is the number-one ornamental fish species in Spermonde Archipelago and large numbers of it and its host anemones are collected and traded. This species is also the most traded ornamental fish in Bali (Reksodihardjo-Lilley and Lilley, 2007). Even though marine ornamental resources in Spermonde Archipelago have been exploited since the beginning of the 1980s (Whitten et al., 2002), only a rather small number of local fishermen are involved in the fishery. However, even though the number of fishermen is comparably small and their activity is spread over the entire archipelago, a fishery targeting almost exclusively *A. ocellaris* and its host anemones can have a negative impact. In addition, although *A. ocellaris* is a popular fish species among aquarists, the price of this fish is rather low. A low price of anemonefishes is also observed in the Philippines (Shuman et al., 2006). Due to the low price that fishermen receive from middlemen, the fishermen spend a huge effort to collect as many anemonefish as possible in order to increase their income.

The trade network persists because of a mutual interdependence between middlemen and fishermen. A similar relationship also exists in India (Sathiadhas and Panikkar, 1992) and Malaysia (Merlijn, 1989). However, the trade network in the Spermonde Archipelago is more complex due to the mutual interdependence between patron and client (*Punggawa-Sawi*), which is also present in other fishery activities in the region (Schwerdtner Máñez and Ferse, 2010). The client-patron relationship on the islands is the first part of the chain that connects the natural resources with the worldwide trade. The *Punggawa* (patron) usually provides the initial capital to start the fishing activity as a debt under a special agreement with the *Sawi* (client). Such agreements stipulate that what is caught by the *Sawi* should only be sold to the *Punggawa*. This informal relation is detrimental for the fishermen, because they cannot increase the low price received for the marine ornamentals.

Poverty and low educational level add to the problems of the marine ornamental fishery in this area. Even though the monthly expenses per person are above the official Indonesian poverty line of € 17.6 (Rp 211,726) per capita and month (BPS 2010), they are very low. In remote islands, such as Sarappo Keke and Papandangan, where marine ornamental fishery generates a high percentage of the income of marine ornamental fishermen, possibilities for an alternative livelihood are rather limited. Fishermen from islands that are closer to the mainland, e.g. Barrang Lompo, have more opportunities to find an alternative livelihood, because of regular boat transport to Makassar. However, the educational level of marine fishermen family members was generally very low. Most of them only finished elementary school and none of the fishermen's family members had a higher education, which makes it very difficult for them to find a job outside the fishery. Due to the lack of education, most fishermen do not have much knowledge on the impact of their fishing activities on the environment.

2.5.2 Impact of the marine ornamental fishery

The present study shows that the densities of both *A. ocellaris* and its host anemones were significantly lower in HE reefs than LE reefs. This is probably caused by the high fishing activity for the marine ornamental trade. A comparable study in the Philippines showed a negative impact of marine ornamental fishing on the density and size of both anemones and the anemonefish *A. larkia* (Shuman et al., 2005). A significant reduction in the density of aquarium fish at collection sites was also observed in Hawaii (Tissot and Hallacher, 2003). Aquarium trade affects both the target species (e.g. decreased in species diversity, abundance, and size structure) and the reef community (e.g. reef living space, increased fish parasites as a result of removal cleaner fish, increased in algal coverage as a result of removal herbivorous fish) in the Great Barrier Reef, Australia (Whitehead et al., 1986). In addition, other phenomena such as bleaching events have a negative impact on the fitness of anemones, due to the loss of its symbiotic zooxanthellae, which eventually die. In turn, this negatively affects anemonefish densities, as observed in the Great Barrier Reef (Jones et al., 2008).

A decrease in anemone densities may have great influence on the possibility of anemonefish larvae to find a suitable habitat in which to settle. Availability of suitable host anemone controls the abundance and distribution of anemonefish, e.g. at different reef zones in Papua New Guinea (Elliott and Mariscal, 2001). Locations with high availability of suitable host sea anemone tend to have higher densities of anemonefish (Richardson, 1999). Dispersal of *Amphiprion ocellaris* might be more restricted than in other anemonefish, because it utilises on only three species of host anemones. For instance, *A. clarkii* is associated with 10 anemone species (Fautin and Allen, 1992) and therefore has a higher probability to find a suitable host. Since *A. ocellaris* imprints itself olfactorily to its host anemone *Heteractis magnifica* (Arvedlund and Nielsen, 1996), the disappearance of anemones could disturb their

olfactory cues and increase their mortality when they fail to find a suitable host anemone.

The present study shows that anemones were occupied by up to eleven individuals of *A. ocellaris*, which was higher than the previously observed 4–5 individuals (Moyer and Nakazono, 1978). The large group size might interfere with the settlement strategy of post-larval clownfish, which prefer to settle in anemones containing smaller resident groups (Mitchell, 2005). This may suggest that some highly exploited reefs had a lack of host anemones, as indicated by the lower density at HE compared to LE. However, at several sites of both exploitation categories, unoccupied anemones were observed (Table 1). These unoccupied anemones could either indicate that all resident clown anemonefish were collected or that larvae had not settled in this host. Due to their symbiotic relationship, the removal of all fish from one anemone might impact the reproduction, growth rate, and the survival of the sea anemone. It was shown that anemones hosting *Amphiprion* grow nearly three times faster than anemones without and that the asexual reproduction of anemones was also enhanced by their presence (Holbrook and Schmitt, 2005).

Extensive harvesting of all size classes at many reef sites in Spermonde Archipelago caused body size reduction. The maximum total length of anemonefish at the island with high exploitation (69 mm) was significantly smaller than at the island with low exploitation (91 mm). However, the maximum total length of anemonefish in both areas was lower than that reported in unexploited populations (110 mm; Allen, 1991). This suggests that marine ornamental fishery has a negative impact on the size of anemonefish even at reefs with low exploitation. A similar result was found in *Amphiprion clarkii*, which were larger in protected reefs than non-protected reefs (Shuman et al., 2006). Due to the decrease in body size, fecundity decreases in highly exploited populations (Birkeland and Dayton, 2005). Size increase of the male would not necessarily increase the reproductive success, but a larger female can produce more eggs than a smaller one (Fricke and Fricke, 1977).

Declining density and decreasing body size are the most revealing and observable impacts of overfishing on target populations, which potentially change the social structure and reproduction system in anemonefish. The queuing system in anemonefish within an individual host anemone forces each individual to stay in its rank according to the actual size ratio (Buston and Cant, 2006), even though some non-breeders within an anemone evict their subordinates to compete for reproduction (Buston, 2003b). In captive condition the functional male of *A. ocellaris* requires about six months to change sex and to attain breeding size when the female is removed from the colony and the largest of the non-breeding individuals simultaneously becomes the functional male. The new breeding pair needs about 12 to 18 months to spawn (Madhu et al., 2010). This shows that the removed breeding pair cannot easily be replaced.

2.5.3 Implications for management and conservation

Indications for over exploitation of anemonefish and its host anemones were revealed in the current study. Consequently, there is a potential for conflicts and social tensions among fishermen as a result of declining and over-fished target populations (Pomeroy et al., 2007). Although the number of fishermen who are engaged in the ornamental fish trade is small (about 3,000 in Spermonde Archipelago), signs of overexploitation are already visible and some populations of *A. ocellaris* and its hosts may collapse. Therefore, a proper management plan and regulations for the marine ornamental fishery are urgent in order to implement a sustainable marine aquarium trade in the region. Since the ecological knowledge of fishermen in Spermonde Archipelago is low, instilling them an understanding of the biology of their target species is a key factor to facilitate a sustainable use and conservation of the species.

The establishment of marine reserve is a well-known strategy for a sustainable use and conservation of coral reef resources (Botsford et al., 2003). For instance, anemonefish and their host sea anemones abundances show an increase within a no-take zone (Scott et al., 2011). A recent study

showed limited connectivity of *Amphiprion ocellaris* populations at the mid-shelf region of the Spermonde Archipelago (Timm and Kochzius, 2008) which predicts a high self-recruitment on the region. In addition, other anemone fishes have shown a variation of self-recruitment ranged from 30–60 % (Planes et al., 2009; Jones et al., 2005; Pinsky et al., 2010). These facts have implications for the management and conservation of this exploited species. High levels of self-recruitment imply that the populations are more vulnerable to fishing activity (Thorrold et al., 2001). Even though these regulations are important initiatives, difficulties might be encountered in managing and controlling them under local circumstances (White, 1986; Wood, 2001b; Glaser et al., 2010). An example in Spermonde Archipelago is the island Badi, which is officially protected since 2006 (Prayudha and Petrus, 2008). This marine protected area (MPA) is not properly working, because newly established MPAs are not well communicated and most local people are not informed about the protection status (Glaser et al., 2010). Anyway, most of the reefs in Spermonde Archipelago are not protected by an MPA or other management measures.

Even though the present study did not investigate all issues concerning marine ornamental fishery impacts in Spermonde Archipelago, the results can serve as a baseline for the management of *A. ocellaris* and its host anemones. While further studies are still required to gain a better understanding of the impact of ornamental fishery on target populations, the present study suggests the following management strategies. First, the breeding pair, typically the two-largest individual in an anemone, should not be collected. Since this is difficult to control, a maximum catch size of 50 mm should be additionally implemented. Fishermen should only collect the smaller sub-ordinate, because the removal of non-breeders does not affect the survival, growth, and reproductive success of breeding pairs (Buston, 2004). Nevertheless, if no breeding pair is present in an anemone, none of the juveniles should be collected to allow them to become reproductive adults. Second, the implementation of MPA network

consisting of no-take areas is recommended, which should take into account their limited connectivity (Timm and Kochzius, 2008). Information on the proportion of self-recruitment is needed to establish a self-sustaining MPA network (Almany et al., 2007). Since only a small part of the population in Spermonde Archipelago is engaged in marine ornamental fishery and since it contributes only a minor part to the income, not many people will be affected by restrictions. However, alternative livelihood options have to be investigated for islands where ornamental fishery contributes a high percentage to the livelihood, such as Sarappo Keke (84 %). Third, a regular monitoring of the *A. ocellaris* population and its host anemones is necessary to control their status. It is also important that at least middlemen are obliged to keep proper records on the amount and size of collected specimens.

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Chapter 3

Fisheries-induced loss of genetic diversity in clown anemonefish (*Amphiprion ocellaris*) island populations of the Spermonde Archipelago, Indonesia



◀ A colony of *Amphiprion ocellaris* on their host anemone *Heteractis magnifica* at Spermonde Arcipelago

Chapter 3

Fishery-induced loss of genetic diversity in clown anemonefish (*Amphiprion ocellaris*) island populations of the Spermonde Archipelago, Indonesia

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Keywords

Exploited species | Phenotypic diversity | Allelic diversity | Genetic variation | Fishery management | Coral triangle

3.1 Abstract

Clown anemonefish *Amphiprion ocellaris* populations in Spermonde Archipelago, which is one of the main collection sites for ornamental fishery in Indonesia, are currently at threat from overfishing, potentially leading to a reduction in population size and genetic variability. Loss of genetic diversity can reduce the adaptability, population persistence, and productivity of the targeted species. Therefore, a study investigating the genetic diversity and its potential correlation to population densities of *Amphiprion ocellaris* was conducted in the region. Two islands were chosen as study sites, Barrang Lompo and Samalona, differing in the degree of exploitation. Population densities in Samalona were threefold higher than in Barrang Lompo ($p = 0.005$), which were obtained using underwater visual census with free-swim surveys in the period May-June

2009. A total of 364 tissue samples of *A. ocellaris* were collected from the two islands under study during October-November 2008 and May-June 2009 to be used for the genetic analysis of eight polymorphic microsatellite loci. A weak but significant genetic difference (F_{ST}) between the two populations was found (0.065 ± 0.048 , $p = 0.01$), as well as high diversity measurements in both populations (mean HE and $D \sim 0.80$). The heterozygote deficit at Barrang Lompo was higher than at Samalona, but no significant difference between the island populations was found ($p > 0.05$). A low inbreeding coefficient of both islands was estimated (mean 0.067 ± 0.125 at Barrang Lompo and mean 0.042 ± 0.123 at Samalona). The numbers of alleles, private alleles, and allelic richness in Samalona were 4% significantly higher than in Barrang Lompo. The number of private alleles found in Samalona was significantly higher than in Barrang Lompo ($p < 0.05$). The allelic richness was significantly different between the two islands ($p < 0.05$), and it was positively correlated with fish density ($p < 0.05$). These results indicated that the ornamental fishery of *A. ocellaris* at the archipelago caused a loss of genetic diversity. Appropriate strategies (e.g. quota, marine reserves) should be implemented in order to manage this valuable resource.

3.2 Introduction

Many marine fish populations are currently impacted due to fishing activities and environmental changes. The most obvious impacts of excessive fishing on marine fish populations are changes in size and age structure (e.g., Trippel 1995; Cardinale and Modin 1999; Ottersen et al. 2006), and reduction in population density (Gaggiotti and Vetter 1999). Due to exploitation, many stocks are depleted and some species are even endangered (e.g., Musick et al. 2000; Hutchings and Reynolds 2004; Dankel et al. 2008). The excessive fishing and environmental changes are not only affecting the spatial distribution and structure of populations, but also causing changes in their genetic diversity (e.g., O'Brien 1994; Heino and Godø 2002; Reusch et al. 2005). The reduction of genetic diversity

might be present long before an effect is actually visible, leading to long-term implications, such as an increased genetic drift augmenting the loss of variability and the ability of adaptation (Spielman et al. 2004, Hauser et al. 2002). A reduction in population size most probably leads to loss of genetic variability (Shaffer 1981; Frankham 1995).

A number of studies revealed a loss of genetic diversity in marine fish due to exploitation, for example, *Hoplostethus atlanticus* (Smith et al. 1991) and *Pagrus auratus* in New Zealand (Hauser et al. 2002). In addition, loss of genetic diversity, associated with high mortality, was found during the recruitment phase of *Dascyllus aruanus* in Moorea (Pini et al. 2011).

More than one thousand marine fish species were traded globally in the period from 1997 to 2002, of which damselfish (Pomacentridae) comprised about 47 %. The clown anemonefish *Amphiprion ocellaris* is the number one fish species and Indonesia is one of the world's leading exporters (Wabnitz et al. 2003). Intensive fishing on *A. ocellaris* in Spermonde Archipelago caused a decrease in density and reduction in body size (Madduppa et al. submitted). The situation may lead to a loss of genetic diversity in *A. ocellaris*.

Due to their biological and behavioural characteristics, the clown anemonefish *Amphiprion ocellaris* is vulnerable to intensive fishing. They have an obligate symbiosis with only three different host anemones (*Heteractis magnifica*, *Stichodactyla gigantea*, and *S. mertensii*). Each anemone is usually inhabited by two to six individuals, where the two largest individuals in the colony are the breeding pair and others remain non-breeders (Fricke and Fricke 1977). They are characterised by a protandrous monogamy (i.e. only one breeding pair lives in a host anemone, and males change sex to become females). *Amphiprion ocellaris* has two phases in its life cycle, a dispersing larvae (8–10 days in captivity, Gopakumar et al. 2009) and sedentary adults. *Amphiprion ocellaris* prefers to settle in smaller groups in order to avoid long breeding queues, maximising their likelihood of attaining social dominance (Mitchell

2005). All individuals will follow the social-rank rule within one anemone by adjusting their size and growth rate (Buston 2003), and the social structure only changes when one individual dies or is removed, after which some time is required for the reproductive male anemonefish to change sex to become the reproductive female (Fricke and Fricke 1977, Madhu et al. 2010; Hattori 1991). Some of their host anemone species (e.g., *Heteractis magnifica* and *Stichodactyla* spp.) are collected and traded in Spermonde Archipelago (Madduppa et al. submitted), which reduces the available habitat for new recruits.

The current study was conducted to investigate the impact of marine ornamental fishing on the genetic diversity of *Amphiprion ocellaris* by using eight microsatellite loci. The present study provides baseline data on the genetic diversity in *A. ocellaris*, aiming to facilitate a better understanding of the impact of marine ornamental fishery on coral reef fishes.

3.3 Materials and method

3.3.1 Study sites

Spermonde Archipelago consists of about 150 islands and is located in the Makassar Strait in the southwest of Sulawesi, in the centre of the Indo-Malay Archipelago (Tomascik et al. 1997). The region is part of the Coral Triangle (Briggs 2009) and is considered to be the world's centre of marine biodiversity (Allen and Werner 2002; Allen 2008; Veron et al. 2009), but is also the most threatened coral reef system in the world (Bryant et al. 1998; Burke et al. 2006). Habitat degradation occurs in the area due to destructive fishing activities (Pet-Soede and Erdmann 1998) and artisanal fishing and harvesting for the marine ornamental industry adds additional pressure (Erdmann 1995; Pet-Soede et al. 1999; Pet-Soede et al. 2001).

The present study was conducted in Barrang Lompo (5°02'52.07"S, 119°19'45.25"E) and Samalona (5°07'30.48"S, 119°20'36.48"E). These two islands are located in the mid-shelf of the Spermonde Archipelago,

close to the city of Makassar. Samples were collected at five sites in Barrang Lompo and four sites in Samalona (Fig.3.1, Table 3.1). About 5000 people are living on Barrang Lompo and only about 100 on Samalona. Coral reefs in Barrang Lompo have been impacted by bombing and local sewage pollution, while in Samalona the impacts have come from anchor damage and pollution from the city (Edinger and Risk 2000). Therefore, the number of coral genera decreased by about 25 % from 1985 to 1995 at both islands (Edinger et al. 1998).

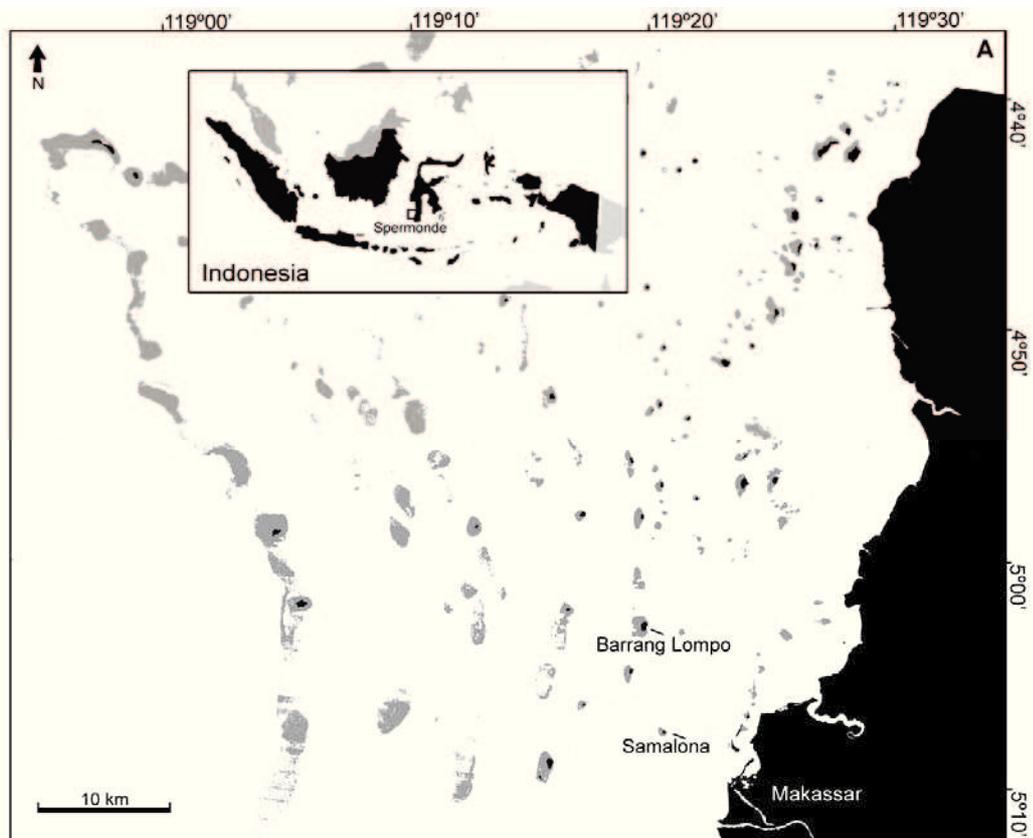


Fig. 3.1: Study sites: Barrang Lompo and Samalona are located in the midshelf of the Spermonde Archipelago.

3.3.2 Fish survey

Amphiprion ocellaris was counted using the underwater visual census technique during free-swim surveys in an approximately 5m-wide area for about 1.5–2 hours for each survey at a depth of 1–11m by scuba diving. Research was carried out during the period May-June 2009. GPS positions

were noted at each survey start and end point in order to estimate the length of each transect. This technique has been successfully used to census the population of anemone fish in larger areas in previous studies (e.g., Shuman et al. 2005, Jones et al. 2008).

3.3.3 Sampling and microsatellite analysis

A total of 364 tissue samples of *A. ocellaris* were collected in Barrang Lompo and Samalona during October-November 2008 and May-June 2009. All individuals (adults, juveniles and recruits) in a colony were caught with two aquarium nets and the caudal fin was clipped to obtain a tissue sample. Subsequently they were released into their host anemones. The samples were preserved in 96 % ethanol and finally stored at 4 °C.

Genomic DNA was extracted with the NucleoSpin Tissue Extraction kit (Macherey-Nagel, Germany), following the manufacturer's guidelines. All DNA extracts were analysed by gel electrophoresis to monitor DNA quality prior to PCR (polymerase chain reaction) amplification of microsatellite loci. GeneRuler™ 1kb DNA Ladder Plus (Applied Biosystems) was used as a reference. PCRs were carried out in a total of 25 µl volume, containing 2.5 µl of 10x PCR buffer, 3 µl of 25 mM MgCl₂, 1 µl 2 mM of each dNTP, 1 µl of each 10 mM primer forward and reverse, 0.1 µl (5 unit/µl) of Taq polymerase (F100L Taq DNA), and 1 µl (1-10 ng) genomic DNA. PCRs were performed in a Tprofessional Thermocycler (Biometra) and a MasterCycler EP (Eppendorf) with the following thermo-profile: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s as denaturing step, 50–65 °C for 30 s as annealing step (see Table 3.2 for annealing temperature at each locus), 72 °C for 1 min for polymerisation, and finally 72 °C for 2 min. Eight polymorphic microsatellite loci were utilised: two isolated from *A. clarkii* (AC137 and AC1578, Liu et al. 2007), four from *A. percula* (Cf39, Cf29, Cf42, and Cf9, Buston et al. 2007), and two from *A. polymnus* (45 and 120, Quenouille et al. 2004). The PCR products were diluted in pure water prior to fragment analysis. Dilution factors were determined empirically for each locus, and ranged

from 1:5 to 1:30. For fragment analysis, 1 μ l of diluted PCR product was combined with 8.85 μ l HiDi™ formamide and the 0.15 μ l GENESCAN LIZ-500 size standard (Applied Biosystems). Microsatellite fragments were size fractionated using an Applied Biosystems Inc. (ABI) 3730 48 capillary sequencer with 50 cm capillary length. Allele sizes were determined and corrected with PEAK SCANNER v1.0 (Applied Biosystems) and GENEMARKER v1.85 (SoftGenetics GeneMarker). Hardy-Weinberg equilibrium (HWE) exact tests and loci combinations for linkage disequilibrium with the Markov chain methods were conducted using GENEPOP on the web (Raymond and Rousset 1995; Rousset 2008). Possible presence of null alleles was tested using the software MICROCHECKER (Van-Oosterhout et al. 2004).

3.3.4 Data analysis

Three measures of the genetic diversity were used: gene diversity, heterozygosity, and number of alleles per locus, which were analysed per sub-site at each island. The number of alleles per locus, observed and expected heterozygosities (Nei 1973), and allele frequencies were calculated with the program CERVUS 3.0 (Marshall et al. 1998). The value of the heterozygosity may range from zero (no heterozygosity) to nearly 1.0 (for a system with a large number of equally frequent alleles). Heterozygote deficit was calculated as $HD = (H_o - H_e)/H_e$. Private alleles are defined as alleles observed in only one population. Gene diversity (the proportion of polymorphic loci across the genome), and allelic richness (mean number of alleles per locus) were calculated with the program FSTAT 2.9.3 (Goudet 1995). F-statistics were calculated using FSTAT in order to detect non-random mating within populations (F_{IS}) and genetic differentiation between the two island populations (F_{ST}) (Weir and Cockerham 1984). The F_{ST} ranges from 0 (no genetic divergence) to 1 (distinct subpopulations), where a F_{ST} value range of 0.00-0.05 indicates that subpopulations have little genetic differentiation, 0.05-0.15 indicates moderate genetic differentiation, 0.15-0.25 indicates high genetic

differentiation, and >0.25 indicates strong genetic differentiation (Wright 1978). F_{IS} value ranges from -1 (no inbreeding) to +1 (complete identity). The software CONVERT 1.3.1 (Glaubitz 2004) was used to anticipate different types of input file formats for the various programs used for the analysis.

Density data for fish were standardised as the number of specimens per 100 m². Density data, observed heterozygosity, heterozygote deficit, F_{IS} , gene diversity, and allelic richness, as well as number of private alleles were compared between the two islands, and tested for significance using the t -test. For unequal variances, the Welsh t -test was used. Linear regression analysis was used to identify a possible correlation between fish density and allelic richness. Here, the nine sites at the two islands were treated as replicates. The statistical analysis was conducted in STATISTICA 7 (Statsoft 2004).

3.4 Results

3.4.1 Population size

The ecological survey conducted in 2009 revealed that the density of *A. ocellaris* was significantly higher at Samalona than at Barrang Lompo (t -test, $t=4.02$, $d.f.=7$, $p=0.005$). The mean density of *A. ocellaris* at Barrang Lompo was 1.60 ± 0.71 ind/100m² (mean \pm SD), while it was more than twice as high in Samalona (mean \pm SD, 3.96 ± 1.06 ind/100m²; Table 3.1).

Table 3.1: Study sites : Geographical coordinates, length of free swims (m), survey area (m²), *A. ocellaris* density (fish/100m²), and the survey period.

| Sites, sub-sites | Geographical coordinates | Length of free swims (m) | Survey Area (m ²) | <i>A. ocellaris</i> density (ind/100m ²) | Time of Survey |
|------------------|------------------------------|--------------------------|-------------------------------|--|----------------|
| Barrang Lompo: | | | | | |
| West | S 05° 02.541' E 119° 19.355' | 763 | 3815 | 1.363 | May 2009 |
| Southwest | S 05° 03.324' E 119° 19.276' | 293 | 1465 | 1.775 | May 2009 |
| North | S 05° 02.507' E 119° 19.571' | 677 | 3385 | 0.679 | May 2009 |
| South | S 05° 03.317' E 119° 19.490' | 395 | 1975 | 2.633 | May 2009 |
| East | S 05° 03.280' E 119° 19.836' | 383 | 1915 | 1.567 | May 2009 |
| Mean | | 502 | 2511 | 1.603 | |
| ± SD | | 205 | 1025 | 0.707 | |
| Samalona: | | | | | |
| West | S 05° 07.010' E 119° 20.006' | 220 | 1100 | 4.818 | June 2009 |
| North | S 05° 07.009' E 119° 20.007' | 560 | 2800 | 2.643 | June 2009 |
| East | S 05° 07.005' E 119° 20.009' | 470 | 2350 | 4.809 | June 2009 |
| South | S 05° 07.011' E 119° 20.009' | 230 | 1150 | 3.565 | June 2009 |
| Mean | | 370 | 1850 | 3.959 | |
| ± SD | | 171 | 857 | 1.056 | |
| <i>t</i> test: | | | | | |
| | <i>t</i> | | | 4.017 | |
| | d.f. | | | 7 | |
| | <i>p</i> -value | | | 0.005 | |

3.4.2 Microsatellite properties, genetic variability, and differentiation

Heterozygosity deficiency showed that 1 of 8 loci departed from HWE (Table 3.2). The presence of null alleles in locus Cf39, as detected with the programme MICROCHECKER, was possible cause for departure from HWE. However, all loci were used in the further analysis. The 28 loci pairwise combinations of genotypic disequilibrium indicate that genotypes of *A. ocellaris* individuals at eight microsatellite loci from each population are independent.

Table 3.2: Measures of genetic variability over eight microsatellite loci from pooled data population of Barrang Lompo (5 sites) and Samalona (4 sites). Given here: sample size (n), expected heterozygosity (H_E), observed heterozygosity (H_o), number of alleles (N_a), number of private alleles (N_a private), allelic richness (A), and gene diversity (D). Asterisk indicates locus departure from HWE after sequential Bonferroni correction ($P < 0.01$).

| Locus | Anneal. Temp. | Barrang Lompo (N=88) | | | | | | | | Samalona (N=276) | | | | | | | |
|--------|---------------|----------------------|-------|-------|----------|-------|-------|-------|-------|------------------|-------|-------|----------|-------|-------|-------|--------|
| | | H_E | H_o | N_a | N_{ap} | A | D | HD | Fis | H_E | H_o | N_a | N_{ap} | A | D | HD | Fis |
| CF9 | 60 | 0.80 | 0.81 | 10 | 0 | 9.98 | 0.797 | 0.01 | -0.01 | 0.80 | 0.82 | 11 | 1 | 11 | 0.800 | 0.02 | -0.019 |
| CF29 | 58 | 0.92 | 0.98 | 18 | 1 | 17.95 | 0.917 | 0.07 | -0.07 | 0.90 | 0.97 | 21 | 4 | 21 | 0.900 | 0.07 | -0.075 |
| CF39 | | 0.95* | 0.68 | 32 | 2 | 31.92 | 0.949 | -0.28 | 0.285 | 0.95* | 0.63 | 37 | 7 | 37 | 0.947 | -0.33 | 0.331 |
| CF42 | 55 | 0.93 | 0.96 | 25 | 1 | 24.89 | 0.930 | 0.03 | -0.03 | 0.93 | 0.94 | 30 | 7 | 30 | 0.931 | 0.00 | -0.004 |
| 45 | 62 | 0.67 | 0.52 | 12 | 0 | 12.00 | 0.675 | -0.22 | 0.225 | 0.58 | 0.57 | 14 | 1 | 14 | 0.580 | -0.01 | 0.013 |
| 120 | 62 | 0.54 | 0.50 | 5 | 0 | 4.98 | 0.539 | -0.07 | 0.073 | 0.59 | 0.59 | 9 | 4 | 9 | 0.592 | 0.00 | -0.004 |
| AC137 | 58 | 0.92 | 0.92 | 20 | 1 | 19.89 | 0.923 | 0.00 | 0.003 | 0.93 | 0.91 | 30 | 12 | 30 | 0.926 | -0.01 | 0.015 |
| AC1578 | 53 | 0.79 | 0.73 | 8 | 0 | 8.00 | 0.792 | -0.08 | 0.082 | 0.81 | 0.78 | 9 | 1 | 9 | 0.810 | -0.03 | 0.033 |
| Mean | | 0.82 | 0.76 | 16 | 1 | 16.20 | 0.815 | -0.07 | 0.067 | 0.81 | 0.78 | 20 | 5 | 20.13 | 0.811 | -0.04 | 0.042 |
| ± SD | | 0.15 | 0.19 | 9 | 0.74 | 9.17 | 0.146 | 0.12 | 0.125 | 0.15 | 0.16 | 11 | 3.89 | 11.01 | 0.149 | 0.12 | 0.123 |

The average observed and expected heterozygosity showed high values in the two populations H_O (mean \pm SD) = 0.76 ± 0.19 and H_E (mean \pm SD) = 0.82 ± 0.15 at Barrang Lompo; H_O (mean \pm SD) = 0.78 ± 0.16 and H_E (mean \pm SD) = 0.81 ± 0.15 at Samalona). The number of alleles, the number of private alleles, and allelic richness found in Samalona was 4 % higher than in Barrang Lompo. Gene diversities of both populations were similar (~ 0.8). Heterozygote deficit in Barrang Lompo (HD = -0.07 ± 0.12 , mean \pm SD) was slightly higher than in Samalona (HD = -0.04 ± 0.12 , mean \pm SD). The inbreeding coefficient (F_{IS}) values in Barrang Lompo ranged between -0.065 for locus CF29 and 0.285 for CF39 (0.067 ± 0.125 , mean \pm SD), which was slightly higher than the values observed in Samalona, ranging from -0.075 for locus CF29 to 0.331 for CF39 (0.042 ± 0.123 , mean \pm SD). Allelic richness (t -test, $t=4.41$, d.f.=7, $p=0.003$) and number of private alleles (t -test, $t=-2.86$, d.f.=14, $p=0.013$) were significantly higher in Samalona. However, gene diversity (t -test, $t=-1.14$, d.f.=7, $p=0.291$), observed heterozygosity (H_O) (t -test, $t=0.58$, d.f.=7, $p=0.578$), heterozygote deficit (HD) (t -test, $t=1.61$, d.f.=7, $p=0.151$), and coefficient of inbreeding (F_{IS}) (t -test, $t=-1.62$, d.f.=7, $p=0.149$) was not significantly different between the two populations (Fig. 3.2). A significant genetic difference (F_{ST}) between the two populations was found (0.065 ± 0.048 , $p=0.01$).

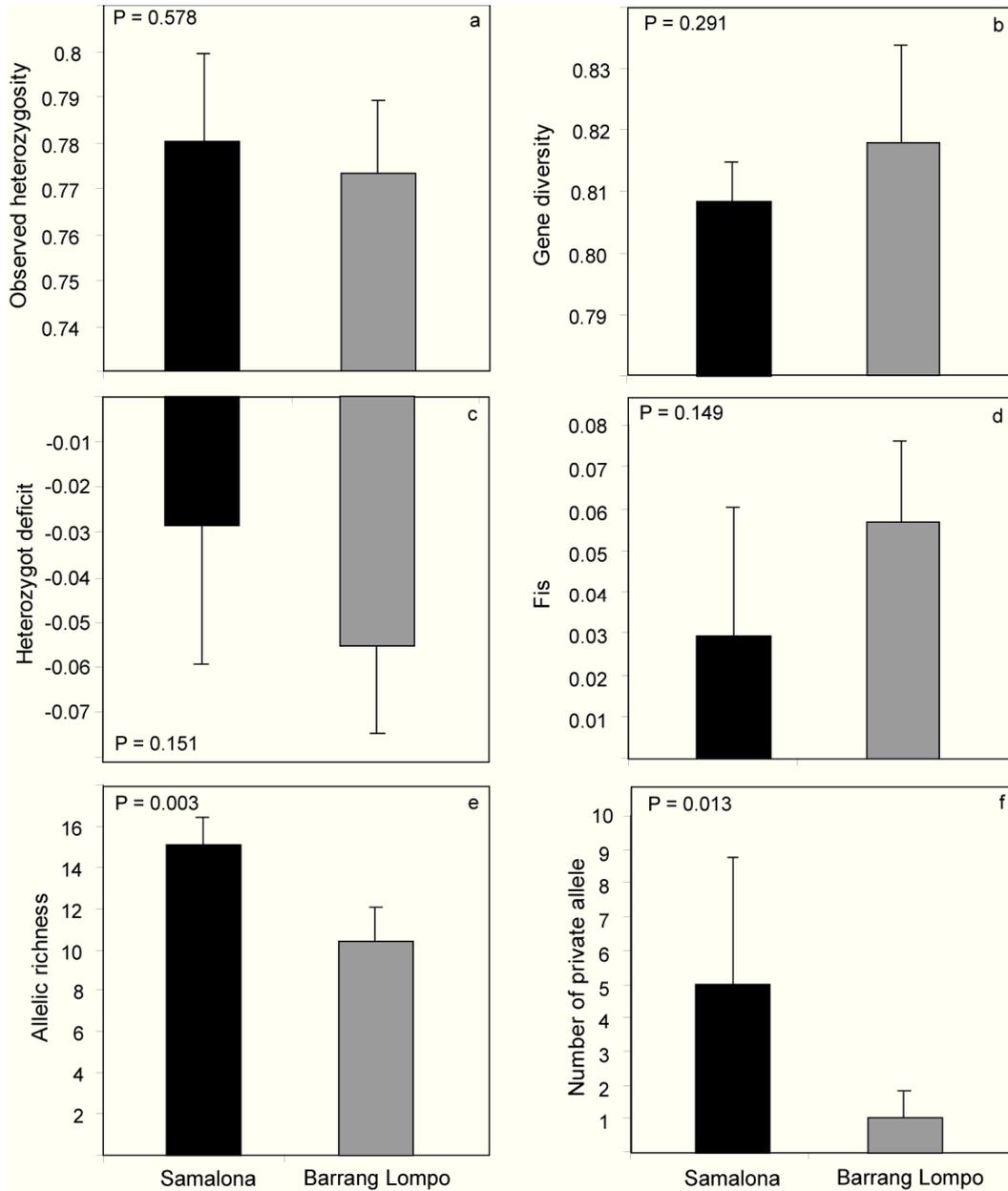


Fig. 3.2: Comparison in fish density, gene diversity, allelic richness, and number of private allele of *Amphiprion ocellaris* between Barrang Lompo and Samalona, given with the significance level.

3.4.3 Population size and its relation to allelic richness

Linear regression analysis showed a positive correlation between fish density and allelic richness ($p=0.03$, $r^2=0.43$, Fig.3.3).

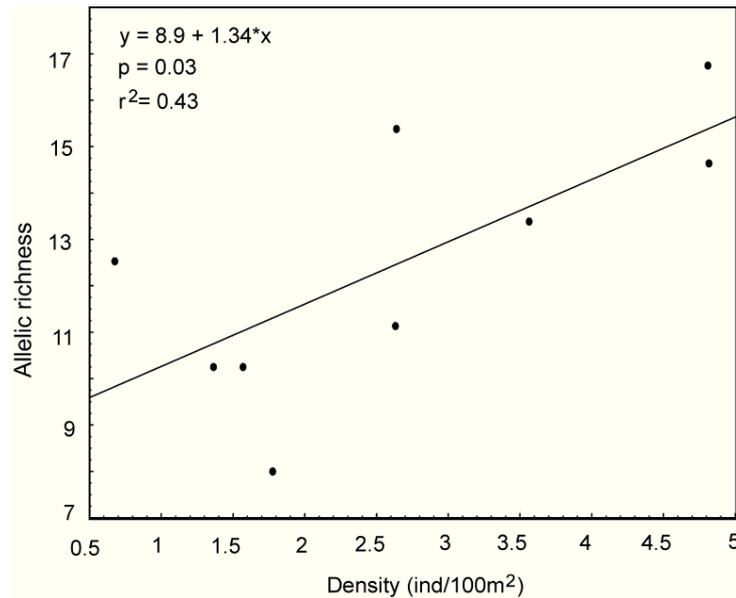


Fig. 3.3: Relationship between allelic richness and fish density, with corresponding coefficient correlation I.

3.5 Discussion

3.5.1 Genetic variability and differentiation between two island populations

A general indicator of the amount of genetic variability and genetic health of a population can be measured by the amount of heterozygosity across all genes in a population. This is because a population needs variation, which is necessary to allow organisms to adapt to an ever-changing environment. Gene diversity and expected heterozygosity between Samalona and Barrang Lompo were not found to be significantly different ($p > 0.05$). Both island populations showed high gene diversity and heterozygosity values (mean H_e and $D > 0.8$). The average expected heterozygosity in *A. ocellaris* for both populations (0.54-0.95) was in a similar range to the previous study on this species conducted by Timm & Kochzius (2008) in the range of 0.54-0.92, and many other marine fish species: 0.16-0.91 in *A. percula* (Buston et al. 2007); 0.91 in *Engraulis japonicus* (Yu et al. 2002); 0.77-0.96 in *Gadus morhua* (Bentzen et al. 1996); 0.75-0.85 in *Epinephelus marginatus* (De Innocentiis et al. 2001); 0.69-0.93 in *Dicentrarchus labrax* (García De León et al. 1997), but

higher than in some other exploited species, such as *Diplodus sargus* populations ($H_e = 0.48 \pm 0.05$) in non-protected areas in the western Mediterranean (Pérez-Ruzafa et al. 2006).

The value of the observed heterozygosity at Samalona was slightly higher than at Barrang Lompo and the heterozygote deficit at Barrang Lompo was higher than at Samalona, but these differences were not significant ($p > 0.05$). Inbreeding in a population could cause a reduction in the heterozygosity and an increase in the homozygosity. However, at both islands no inbreeding effects were revealed, which was indicated by low inbreeding coefficients (mean 0.067 ± 0.125 at Barrang Lompo and mean 0.042 ± 0.123 at Samalona).

In contrast to the heterozygosity, allelic richness and number of private alleles showed significantly higher values in Samalona than in Barrang Lompo. Some studies revealed that allelic richness may be more sensitive to a population bottleneck and environmental stress than heterozygosity (e.g., Allendorf 1986; Petit et al. 1998; Leberg 2002; Pini et al. 2011). A loss of genetic diversity was also found in other exploited species like *Hoplostethus atlanticus* (Smith et al. 1991) and *Pagrus auratus* (Hauser et al. 2002) in New Zealand. In the latter case, a significant decrease in both heterozygosity and the allelic richness over the last 50 years since the onset of exploitation was revealed by seven microsatellite loci. In addition, a loss of allelic richness in surviving populations associated with high mortality was found during the recruitment stage of *Dascyllus aruanus* in Moorea, induced by environmental stress (Pini et al. 2011).

Despite dispersing larvae, *Amphiprion ocellaris* showed a significant genetic differentiation between the two island populations ($F_{ST} = 0.065 \pm 0.048$, $p=0.01$). In such small scale, other anemonefish *Amphiprion clarkii* has significant F_{ST} (<0.028) between any two sites (11%) at the Philippines (Pinsky et al. 2010). A strong genetic population structure was also found at a larger scale in the same species as well as in other coral reef organisms in this region (Kochzius & Nuryanto 2008; Timm and Kochzius 2008; Knittweis et al. 2009; Nuryanto & Kochzius 2009). In

addition, the previous study on these two island populations revealed high self-recruitment in *A. ocellaris* of about 40-60 % (Madduppa et al. in prep.), which indicates that most individuals stay in their native population. The value of F_{ST} revealed in the present study is similar to the study on *Acanthochromis polyacanthus* which has no pelagic larvae ($F_{ST} = 0.0603$, Gerlach et al. 2007), and is higher than found in other marine fish species such as *Ostorhincus doederleini* ($F_{ST} = 0.0189-0.0298$, Gerlach et al. 2007) at the Great Barrier Reef, *Anguilla japonica* ($F_{ST} = 0.002$) in Taiwan (Han et al. 2008), and *Haemulon flavolineatum* ($F_{ST} \approx 0.003$) in Caribbean (Purcell et al. 2006). Theoretically, levels of genetic differentiation are influenced by various factors, which include connectivity, history and size of population (Hauser and Carvalho 2008).

3.5.2 Relationship between population size and genetic diversity

The relative population size in Barrang Lompo was threefold lower than in Samalona ($p = 0.005$). Barrang Lompo and many other reefs within Spermonde Archipelago are already exploited for a long time (Erdmann 1995; Pet-Soede et al. 1999; Pet-Soede et al. 2001). Reefs within the archipelago suffered from destructive fishing, anchor damage and pollution from the city, and the number of coral genera at both islands decreased by about 25 % from 1985 to 1995 (Edinger et al. 1998). A decline in abundance is considered to be the first and most obvious response of a fish population to exploitation (Schumann et al. 2005). Reduced population sizes drive the alteration of genetic and phenotypic variability (review by Marteinsdóttir and Pardoe 2008, Gaggiotti and Vetter 1999), and may cause a loss of genetic diversity and therefore potentially reduce the adaptability, population persistence, and productivity of the targeted species (Hauser et al. 2002).

Increased homozygosity and expression of recessive deleterious alleles are the main consequences of changes in population size (Lande 1988; England et al. 2003). The current study has revealed that allelic richness from eight microsatellite loci and fish density in *A. ocellaris* were positively correlated ($p < 0.05$). In other words, the higher the fish density

the more genetic variability and thus a decrease in fish density due to exploitation can lead to a reduction of genetic diversity. Since many individuals are removed from the population by marine ornamental fishery, there is the potential consequence that some alleles are also removed. Fishing activities, which usually remove the older and bigger individuals, are now potentially removing the most heterozygous individuals (Smith et al. 1991). In addition, due to a reduced population size, genetic drift will increase and alleles will eventually be lost (Garza and Williamson 2001). This should therefore be taken into account when managing marine ornamental fishery in the region, as described below.

3.5.3 Implications for management and conservation

Kenchington et al. (2003) proposed several forms of action to prevent the loss of genetic diversity in marine species, which include: maintaining the number and relative size of the population, maintaining a large abundance of individual populations, and minimising fisheries-induced selection. The clown anemonefish *A. ocellaris* has been heavily exploited at the Southeast Asian countries to meet global demand for the aquarium trade (Wabnitz et al. 2003). It has recently been revealed that the marine ornamental fishery in the Spermonde Archipelago has reduced the density and average size of *A. ocellaris* (Madduppa et al, submitted). In addition, the current study showed a strong indication that *A. ocellaris* at the two studied islands was also impacted at the genetic level, probably by the strong fishing pressure especially in Barang Lompo, which is highly populated.

Based on the gathered information, both the region and the trade in marine ornamental fish need to be managed; otherwise the population of *A. ocellaris* may collapse. The present study supports the proposed implications put forward by Madduppa et al. (submitted) for the management and conservation of the focal species in this region. First, management of the fishing strategy is required, such as leaving the breeding pair in a colony or placing a restriction on the maximum catch

size of 50 mm. This strategy is good for preventing the loss of rare allele from older and bigger individuals. Second, the implementation of marine protected areas (MPAs), which take into account high self-recruitment in *Amphiprion ocellaris* (Madduppa et al. submitted) and the limited connectivity of populations (Timm and Kochzius 2008, Timm et al. 2012). High self-recruitment implies that the populations are more vulnerable to fishing activity (Thorrold et al. 2001). MPAs are an important tool to prevent overexploitation and to ensure the sustainable use of living marine resources (Agardy 1994). In Indonesia MPAs have been proposed for managing marine fisheries (Mous et al. 2005). They could also be an effective tool for protecting the genetic biodiversity, which offers benefits for the conservation of the allelic richness and for preserving rare alleles (Pérez-Ruzafa et al. 2006). Third, a regular monitoring and quota determination of the *A. ocellaris* population are necessary to control their status as a part of the management of the species, which in turn could reduce pressure on the targeted species.

3.5.4 Summary

In conclusion, this study has demonstrated that allelic richness is higher in the population of the less exploited reefs of Samalona than at the high exploited reefs of Barrang Lompo. It seems that allelic richness is more sensitive to the impact of ornamental fishery of *A. ocellaris* in the Spermonde Archipelago than heterozygosity. A positive correlation between allelic richness and fish density was revealed. However, it seems complex to infer the genetic diversity of one particular species regarding population size, because many other factors may also affect genetic diversity, such as population structure (Cherry and Wakeley 2003), population bottlenecks (O'Brien 1994), and natural selection (Charlesworth et al. 1993). Furthermore, additional studies are still needed to be conducted in the region, because widespread exploitation and many more fishing grounds were found within the archipelago. As the number of sites surveyed limited this study, data from additional sites may provide

a more precise picture of the impact of marine ornamental fishery. This kind of research should be continued on a larger scale in order to obtain genetic structure data for *A. ocellaris* and other targeted species across a larger part of the archipelago. With that, the reduction of genetic diversity might be detected at an early stage. Nevertheless, the current results could be used as a baseline status to determine the genetic diversity changes in the future. Meanwhile, the marine ornamental fishery and other fisheries in the Spermonde Archipelago should be managed with an appropriate strategy (e.g. quotas, marine reserves) in order to avoid loss of genetic diversity.

3.6 Acknowledgement

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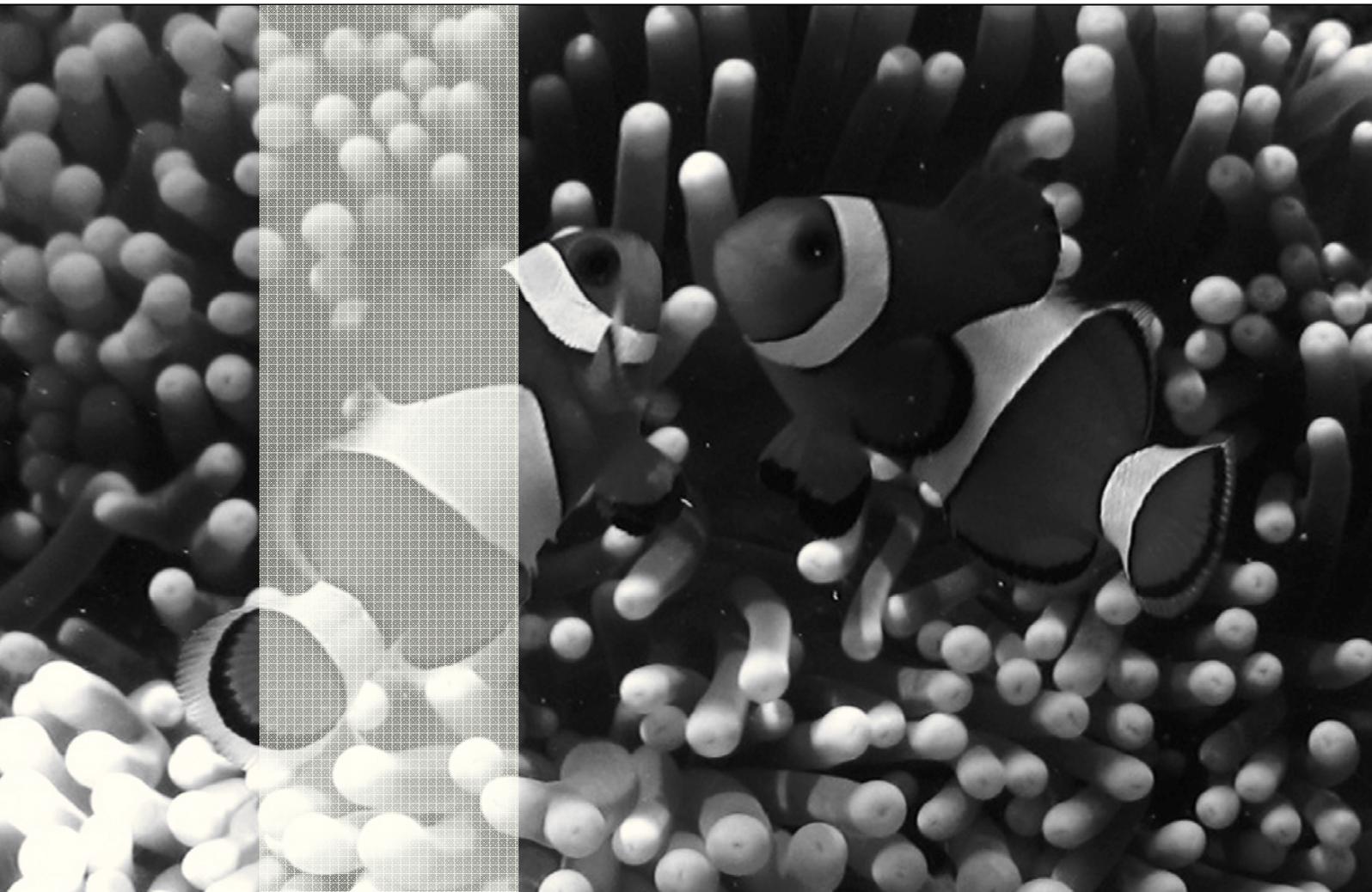
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Chapter 4

High self-recruitment in anemonefish species revealed by microsatellite DNA parentage analysis



◀ A breeding pair of *Amphiprion ocellaris* on their host anemone *Heteractis magnifica* at Spermonde Arcipelago

Chapter 4

High self-recruitment in anemonefish species revealed by microsatellite DNA parentage analysis

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4.1 Abstract

Anemonefish are the most collected fish species for the aquarium trade in the Spermonde Archipelago (Indonesia), but little effort has been made for a sustainable management, urgently needed to prevent overexploitation. An important aspect for the management of living marine resources is the extent of self-recruitment and the connectivity of populations. A DNA parentage analysis using polymorphic microsatellite markers was conducted to investigate self-recruitment, site fidelity, and genetic relatedness in populations of the clown anemonefish *Amphiprion ocellaris* and the pink anemonefish *A. perideraion*. Tissue samples of *A. ocellaris* ($n = 364$) and *A. perideraion* ($n = 105$) were collected at Barrang Lompo Island (high exploitation) and Samalona Island (low exploitation) in Spermonde Archipelago. Specimens were genotyped based on seven microsatellite loci for *A. ocellaris* and five microsatellite loci for *A. perideraion*, and parentage assignment as well as site fidelity were calculated. Self-recruitment of *A. ocellaris* in Samalona (60.7 %) is higher

than for *A. ocellaris* (47.4 %) and *A. perideraion* (44 %) in Barrang Lompo. The estimated self-recruitment for *A. ocellaris* in Samalona was much higher than the reported self-recruitment of the sibling species *A. percula*. Individuals of *A. ocellaris* and *A. perideraion* were leaving their native site in different proportions. However, most of them settled close to their origin site. Considering these facts, the current study may have an implication for management and conservation. Small MPAs, preferably on every island/reef, should ensure that a part of the population is protected to enable replenishment by self-recruitment.

4.2 Introduction

Self-recruitment is related to the number of larvae returning to and settling in their natal population, whereas population connectivity is the linking of distinct populations by individual dispersal or migration. These two aspects are fundamental for the management and conservation of the diversity of living marine resources (Fairweather 1991), management of highly harvested species (Roberts 1997), understanding the population dynamics of marine organisms (Underwood and Fairweather 1989), and improving the design of marine reserves (Almany et al. 2009). Sufficient self-recruitment of marine organisms and their connectivity among populations in marine reserves are believed to prevent local extinction as a result of anthropogenic disturbances such as fishing pressure (Sala et al. 2002). However, determining the degree of self-recruitment and the connectivity in marine organisms such as fish are challenging issues due to the influence of many factors. First, the pelagic larval duration (PLD) varies from days to weeks in fish (Wellington and Victor 1989) and is related to dispersal capability (Lester and Ruttenberg 2005). Second, the early larval stage is potentially influenced by oceanographic processes (Wilson and Meekan 2001) and physical variables, e.g. geographic location and flow variability of ocean currents (Sponaugle et al. 2002). Third, larval behaviour, such as olfactory reef sensing, may be used by

early developmental stages of fish to control their dispersal (Kingsford et al. 2002) and to detect their native habitat (Arvedlund and Nielsen 1996).

Genetic markers are widely used for addressing wildlife management issues in different organisms (Bruford and Wayne 1993; Wright and Bentzen 1994; Ferguson et al. 1995; Sunnucks 2000; Schwartz et al. 2007). Microsatellites are simple repetitive sequences, located throughout the eukaryote nuclear genome (Tautz 1989). Because of their high variability they are widely used for fine-scale ecological studies, such as parentage analysis (Selkoe and Toonen 2006). Polymorphic microsatellite DNA parentage analysis uses genotype data as the basis for relationship reconstruction based on the maximum likelihood method, where juveniles are assigned to the most likely parent from a data set of potential parents (Jones and Ardren 2003). This method has been proven to be a powerful tool for investigating self-recruitment in marine fish (Jones et al. 2005; Almany et al. 2007; Daly-Engel et al. 2007; Planes et al. 2009). A number of studies were able to identify connections among populations by using parentage analysis (Saenz-Agudelo et al. 2009; Saenz-Agudelo et al. 2011). Genetic relatedness of individuals within a group could also give an indication of whether larvae of marine organisms remain in the origin population or disperse to other regions (Buston et al. 2007).

Anemonefish have two very different phases in their lifecycle: sedentary adults are associated to certain host anemones, while larvae are planktonic. The PLD of the clown anemonefish *Amphiprion ocellaris* is 8-12 days and it can live in symbiosis with three anemone species: *Heteractis magnifica*, *Stichodactyla gigantea*, and *S. mertensii* (Fautin and Allen 1994). The pink anemonefish *Amphiprion perideraion* has a PLD of 18 days (Wellington and Victor 1989) and can be associated with four different anemones: *Heteractis magnifica*, *H. crispata*, *Macroactylia doreenis*, and *Stichodactyla gigantea*. These two species are host specialists compared to other anemonefish, such as *A. clarkii*, which can live in symbiosis with ten anemone species (Fautin and Allen 1994). Due to the limited number of host species *A. ocellaris* and *A. perideraion* are

probably limited in their dispersal capability, which increases its vulnerability to high fishing pressure. With 145,000 individuals collected from the wild from 1997 to 2002, *A. ocellaris* is the number-one marine ornamental fish species traded on the global market (Wabnitz et al. 2003). In Spermonde Archipelago (Indonesia), *A. ocellaris* is the most traded marine ornamental fish species as well, and there are already strong indications of overexploitation (Madduppa et al. submitted). Thus, a proper management, including the implementation and effective enforcement of marine protected areas (MPAs) is needed to avoid overexploitation and depletion of this commercially harvested fish species.

A recent study showed limited connectivity of *A. ocellaris* populations across Indonesia as well as among shelf areas in the Spermonde Archipelago, predicting high self-recruitment in the mid-shelf area of the archipelago (Timm and Kochzius 2008). Thus, the present study was performed to investigate the degree of self-recruitment, the genetic relatedness, and site fidelity of *A. ocellaris* and *A. perideraion* populations of two small islands in the mid-shelf region (Barrang Lompo and Samalona) of Spermonde Archipelago by polymorphic microsatellite DNA parentage analysis. Understanding the degree of self-recruitment in these populations and their connectivity to neighbouring populations could directly support the implementation of effective MPA networks, as well as the sustainable management and conservation of this species.

4.3 Materials and methods

4.3.1 Study sites and sampling

The Spermonde Archipelago (South Sulawesi, Indonesia) comprises an area of about 150 islands (Tomascik et al. 1997) and is situated at the south-western tip of Sulawesi in the centre of marine biodiversity, the so-called “coral triangle”. This archipelago is affected by the very strong Indonesian Throughflow (ITF), which connects the Pacific Ocean with the Indian Ocean (Wyrski 1961). This setting potentially enhances the

dispersal of marine organisms in Spermonde Archipelago. On the contrary, interaction between oceanographic processes and larval behavior may enable larvae to stay close to their natal population (James et al. 2002). There are about 50,000 inhabitants in Spermonde Archipelago, which are relying on coral reef resources. Therefore, reefs are under threat from different kinds of anthropogenic activities, such as fishing, including destructive practices, and land-based pollution (Edinger et al. 1998). The present study was conducted at two small islands, Barrang Lompo and Samalona (Fig.1), located in the mid-shelf of Spermonde Archipelago. Barrang Lompo (5°02'52.07" S, 119°19'45.25" E) is located about 13 km west of Makassar, is approximately 19 ha in size and is inhabited by about 5000 people. Its fringing coral reefs (approximate circumference 6 km) have been impacted by dynamite-fishing and local sewage pollution (Edinger and Risk 2000). Samalona (5°07'30.48"S, 119°20'36.48"E) is located about 5 km west of Makassar, has a size of about 2 ha, and is inhabited by about 80 people. This island has a fringing reef with an approximate circumference of 2 km. This island has been developed by local people for small-scale tourism and is therefore relatively protected from destructive fishing activities. However, Samalona's reefs have been impacted by anchor damage and pollution from Makassar (Edinger and Risk 2000).

A total of 364 tissue samples of *A. ocellaris* and 105 tissue samples of *A. perideraion* were collected at the study sites. The reef in Barrang Lompo was divided into five and the reef in Samalona into four sites (Fig.1). In Barrang Lompo (surveyed area: about 12.6 km² at the reef slope), 88 *A. ocellaris* individuals were sampled from 17 anemones October-November 2008: 31 resident adults, 25 sub-adults as well as juveniles, and another 32 juveniles in May 2009. In Samalona (surveyed area: about 7.4 km² at the reef slope), a total of 276 individuals consisting of 164 resident adults, and 112 sub-adults and juveniles were sampled from 83 anemones in May 2009. Of the species *A. perideraion*, 105 individuals were sampled from 35 anemones in Barrang Lompo in May 2009: 41 resident adults and 64

sub-adults as well as juveniles. Every effort was made to ensure that all individuals in each anemone present at the studied islands were sampled.

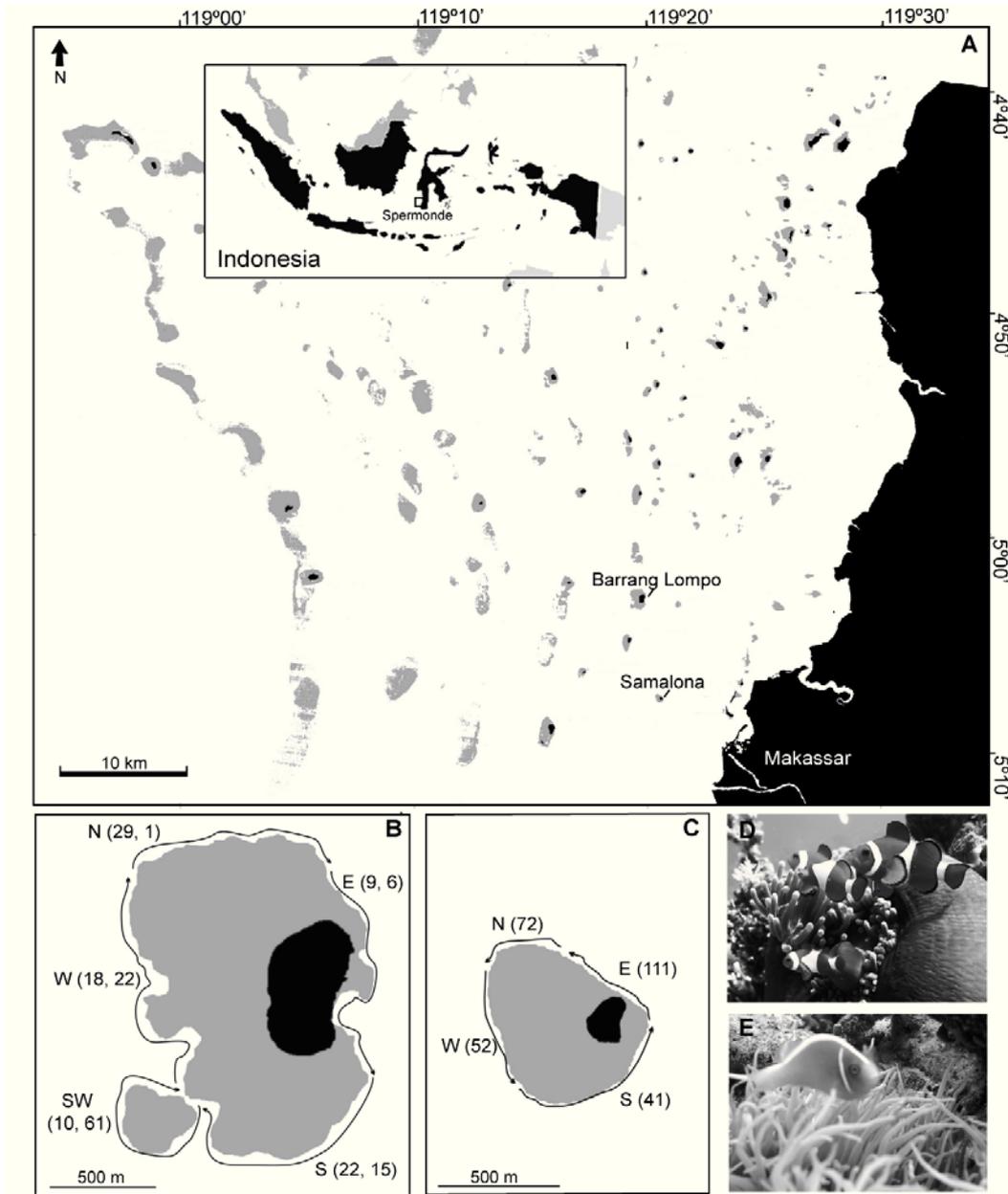


Fig. 4.1: (A) Map of study sites: Barrang Lompo and Samalona in Spermonde Archipelago. Inset: the location of the study sites in South Sulawesi, Indonesia. (B) Figures in brackets indicate number of *Amphiprion ocellaris* and *A. perideraion* samples at samples sites in Barrang Lompo. (C) Figures in brackets indicate number of *A. ocellaris* samples at samples sites in Samalona. N: North, E: East, W: West, SW: Southwest, and S: South. Pictures show a colony of *A. ocellaris* (D) and an individual of *A. perideraion* I in their host anemone. The arrows indicate free swim survey sampling at reef slope.

The sex of individuals was determined by body size, whereat the largest fish was the reproductive female, the second-largest fish was the reproductive male, and all others were non-breeding individuals (Fricke and Fricke 1977; Moyer and Nakazono 1978). Each individual was caught with two aquarium nets while scuba diving. Tissue samples were taken by clipping a piece from the caudal fin and afterwards the specimens were released to their host anemones. Re-sampling can be ruled out, because fin-clipped individuals could be easily identified. Each tissue sample was put into a separate tube. All information regarding the tissue samples (e.g., species, size, date, location, and anemone) was recorded underwater. Tissue samples were preserved in 96 % ethanol after the dive and finally stored at 4 °C in the laboratory until DNA extraction.

4.3.2 DNA extraction, microsatellite amplification and allele sizing

Genomic DNA from *A. ocellaris* and *A. perideraion* was extracted with NucleoSpin Tissue extraction kits (Macherey-Nagel), following the manufacturer's guidelines. All DNA extracts were analysed by gel electrophoresis to monitor DNA quality prior to polymerase chain reaction (PCR) amplification of microsatellite loci. DNA extracts were stored at -20 °C.

PCRs were carried out in a total volume of 25 µl, containing 2.5 µl 10x PCR buffer, 3 µl 25 mM MgCl₂, 1 µl 2 mM each dNTP, 1 µl each 10 mM primer forward and reverse, 0.1 µl (5 unit/µl) Taq polymerase (F100L Taq DNA), 1 µl (1-10 ng) genomic DNA. PCRs were performed in a TProfessional Thermocycler (Biometra) or a Mastercycler ep (Eppendorf) with the following thermo-profile: 94 °C for 2 minutes, followed by 35 cycles of 94 °C for 30 seconds as the denaturing step, 50–65 °C for 30 seconds as the annealing step (the optimal annealing temperature varies between primers, see Table 1), 72 °C for 1 minute for the polymerisation, and finally 72 °C for 2 minutes.

Seven polymorphic microsatellite loci were used for *A. ocellaris*: two from *A. clarkii* (AC137 and AC1578; Liu et al. 2007), three from *A.*

percula (Cf42, Cf29, and Cf9; Buston et al. 2007), and two from *A. polymnus* (45 and 120; Quenouille et al. 2004). For *A. perideraion*, five microsatellite loci were utilised: three from *A. clarkii* (AC137, AC915, and AC1578; (Liu et al. 2007), one from *A. percula* (Cf42; Buston et al. 2007), and one from *A. polymnus* (120; Quenouille et al. 2004). The chosen loci were amplified by PCR with a labelled forward primer containing a 5'-fluorescent dye (FAM or HEX) (Table 1).

PCR products were diluted in pure water prior to fragment analysis. Dilution factors were determined empirically for each locus, and ranged from 1:5 to 1:30. For fragment analysis, 1 µl of diluted PCR product was combined with 8.85 µl HiDi™ formamide and 0.15 µl GENESCAN LIZ-500 size standard (Applied Biosystems). Microsatellite fragments were size fractionated using an ABI 3730 48 capillary sequencer with a capillary of 50 cm length (Applied Biosystems). Allele sizes were determined and corrected with PEAK SCANNER v1.0 (Applied Biosystems) and GENEMARKER v1.85 (SoftGenetics GeneMarker). Three types of repeat motif were found from all loci: a dinucleotide motif (AC137, AC915, AC1578, Cf29, 45 and 120), tetranucleotide motif (Cf9) and di-tetranucleotide motif (Cf42) (Table 1). The program MICROCHECKER was used to detect null alleles and to identify irregularities in the data, including mistyped allele sizes, typographic as well as scoring errors (Van-Oosterhout *et al.* 2004).

Table 4.1: Microsatellites loci used for *Amphiprion ocellaris* and *A. perideraion*. Given here: name of the locus (Locus), repeat motif, annealing temperature (Ann.) for *A. ocellaris* (Ao) and *A. perideraion* (Ap), primer sequences, fluorescence dye, organism, and reference.

| Locus | Repeat motif | Ann. Ao | Ann. Ap | Primer sequences (5'-3') | Dye | Organism | Reference |
|--------|-------------------|------------|------------|---|-----|---------------------------|------------------------|
| Cf9 | Tetranucleotide | 60 | - | F: CTC TAT GAA GAT TTT T R: GTA CAT GTG TTT CCTC | HEX | <i>Amphiprion percula</i> | * |
| Cf42 | Ditetranucleotide | 55 | 53 | F: AAG CTC CGG TAA CTC AAA ACT AAT R: GTC ATC TGA TCC ATG TTG ATG TG | HEX | <i>A. percula</i> | Buston et al. 2007 |
| Cf29 | Dinucleotide | 58 | - | F: TTC TTT ATC CCC TTG TTT ATT TCT AA R: AAG CCT CCT TTC CAA AAC CAC TCA | FAM | <i>A. percula</i> | Buston et al. 2007 |
| 45 | Dinucleotide | 62 | - | F: TCA ACT GAA TGG AGT CCA TCT GG R: CCG CCG CTA GCC GTG ACA TGC AA | FAM | <i>A. polymnus</i> | Quenouille et al. 2004 |
| 120 | Dinucleotide | 62 | 68 | F: TCG ATG ACA TAA CAC GAC GCA GT R: GAC GGC CTC GAT CTG CAA GCT GA | HEX | <i>A. polymnus</i> | Quenouille et al. 2004 |
| AC1578 | Dinucleotide | 53 | 55 | F: CAG CTC TGT GTG TGT TTA ATG C R: CAC CCA GCC ACC ATA TTA AC | FAM | <i>A. clarkii</i> | Liu et al. 2007 |
| AC137 | Dinucleotide | 58 | 55 | F: GGT TGT TTA GGC CAT GTG GT R: TTG AGA CAC ACT GGC TCC T | FAM | <i>A. clarkii</i> | Liu et al. 2007 |
| AC915 | Dinucleotide | - | 58 | F: TTG CTT TGG TGG AAC ATT TGC R: TCT GCC ATT TCC TTT GTT C | HEX | <i>A. clarkii</i> | Liu et al. 2007 |

4.3.3 Summary statistics, test of Hardy-Weinberg equilibrium, and linkage disequilibrium

The total number of alleles per locus, allele frequencies, observed and expected heterozygosities (Nei 1973), and the Polymorphic Information Content (PIC) of each locus (Botstein et al. 1980) were calculated with the program CERVUS 3.0 (Marshall et al. 1998). The categories of PIC values used for microsatellite loci were: (1) highly informative ($PIC > 0.5$), (2) reasonably informative ($0.5 > PIC > 0.25$), and (3) slightly informative ($PIC < 0.25$), following Botstein et al. (1980). Hardy-Weinberg equilibrium (HWE) exact tests and loci combinations for linkage disequilibrium with the Markov chain methods were conducted using GENEPOP on the web (Raymond and Rousset 1995; Rousset 2008). In order to test the null hypothesis of HWE, the probability test was conducted. The alternative hypothesis of heterozygote deficiency was tested with using scores. The null hypothesis of linkage disequilibrium for the diploid case was tested through pairwise comparisons of loci. For all Markov chain methods, parameters used were the default settings for dememorisation number (1000), number of batches (100), and iterations per batch (1000). Significance levels were adjusted with sequential Bonferroni corrections for multiple tests with $P < 0.01$. The software CONVERT 1.3.1 (Glaubitz 2004) was used to obtain the correct file formats for the various programs applied.

4.3.4 Self-recruitment and site fidelity

Analysis of self-recruitment and site fidelity using DNA parentage analysis were conducted with FAMOZ (Gerber et al. 2003). Origin site is defined as a site where juveniles were sampled. Using a likelihood-based approach (Meagher 1986), juveniles were assigned to a single parent or parent pair in order to select the most likely parent from a pool of potential parents (Jones and Ardren 2003). Suitability of this program for such analyses in fish populations was shown in several studies (Jones et al. 2005; Planes et al. 2009; Saenz-Agudelo et al. 2009). In this program, the

exclusion probability (Jamieson and Taylor 1997) is generated, which uses incompatibilities between parents and offspring to reject particular parent-offspring hypotheses (Jones and Ardren 2003). The log-likelihood ratios or logarithm of the odds (LOD) scores were calculated by this program for each parent/offspring association, using microsatellite allele frequencies calculated by CERVUS 3.0. LOD score threshold values for each error type were taken from the intersection of offspring with genotyped parents and offspring generated according to allele frequencies. The parameters for the parent/offspring assignment decision were as follows (Meagher and Thompson 1986): (1) individuals were assigned to the most likely single parent if the LOD score \geq single parent (T_P) and (2) individuals were assigned to the most likely parent pair if the LOD score \geq single parent (T_P) and the LOD score \geq parent pair (T_C). Simulations have to be performed to determine suitable thresholds, error levels, and the impact of scoring errors for each population (Gerber et al. 2000). Therefore, five different error rates were evaluated for all data sets (Table 2). Each error rate was evaluated with 10,000 replicates of simulated offspring, with the assumption of a large random mating population. Finally, to compensate for error in scoring parents or offspring genotypes, the presence of null alleles, and marker mutation (Marshall et al. 1998), an error rate of 0.01 was chosen for parentage analysis for all populations of *A. ocellaris*, and an error rate of 0.001 for *A. perideraion* populations (Table 2). In Barrang Lompo, LOD score threshold values at this error rate for single parent (SP) and parent pair (PP) of *A. ocellaris* were 1.9 and 5.9, respectively. In Samalona, LOD score threshold values at this error rate for single parent (SP) and parent pair (PP) of *A. ocellaris* were 2.5 and 6.7, respectively. For *A. perideraion*, LOD score threshold values at this error rate for single parent (SP) and parent pair (PP) were 2.8 and 7.9, respectively. Unassigned juveniles of *A. ocellaris* to a single parent or parent pair in Barrang Lompo were assigned to potential parents in Samalona, and vice versa. These two data sets were also simulated to obtain suitable LOD

thresholds (Table 2). All tests showed high cumulated exclusion probabilities (> 0.9).

Table 4.2: Evaluation of five different error rates on parentage assignment using: (A) *A. ocellaris* data set of Barrang Lompo and (B) Samalona, (C) *A. perideraion* data set of Barrang Lompo population, (D) *A. ocellaris* data of Samalona (parents) and Barrang Lompo (juveniles) and I Barrang Lompo (parents) and Samalona (juveniles), with the estimation of LOD threshold for single parent (SP) and parent pair (PP), and type I and type II statistical errors, and observed gene flow. Bold numbers indicate the LOD thresholds used for analysis.

| | | Error rates | | | | |
|---------------------------|---|-------------|--------|------------|------------|--------|
| Population | | 0 | 0.0001 | 0.001 | 0.01 | 0.1 |
| Threshold (SP) | A | 0.6 | 0.7 | 2.9 | 1.9 | 0.2 |
| | B | 0.9 | 3.8 | 3.7 | 2.5 | 0.7 |
| | C | 2.5 | 3.3 | 2.8 | 1.7 | 0.5 |
| | D | 0.9 | 3.85 | 3.9 | 2.6 | 0.6 |
| | E | 0.8 | 2.6 | 3.01 | 2.1 | 0.3 |
| Threshold (PP) | A | 8.1 | 8.5 | 8.4 | 5.9 | 0.7 |
| | B | 9.4 | 10.6 | 10.3 | 6.7 | 1.7 |
| | C | 9.6 | 9.3 | 7.9 | 4.7 | 0.9 |
| | D | 9.5 | 10.75 | 10.7 | 6.8 | 2 |
| | E | 10.4 | 10.2 | 8.4 | 6 | 1.3 |
| Type error I (α) | A | 0 | 0.0 | 0.5 | 8.3 | 18.9 |
| | B | -0.0001 | 1.3 | 4.5 | 27.6 | 145.9 |
| | C | 0.19 | 0.3 | 3.4 | 39.4 | 75.2 |
| | D | -0.0012 | 1.1 | 4.4 | 30.3 | 107.3 |
| | E | 2.9 | 0.0 | 0.0 | 7.2 | 67.6 |
| Type error II (β) | A | 7.4 | 15.8 | 9.4 | 12.3 | 1.3 |
| | B | 22.6 | 14.0 | 13.4 | 4.5 | -60.8 |
| | C | 4.69 | 6.97 | 10.42 | -2.45 | -74.16 |
| | D | 22.8 | 13.7 | 13.2 | 4.9 | -33.9 |
| | E | 3.4 | 6.4 | 9.6 | 10.9 | -63.0 |
| Gene flow | A | 0.92 | 0.84 | 0.86 | 0.7 | 0.2 |
| | B | 0.79 | 0.82 | 0.75 | 0.65 | 0.36 |
| | C | 0.72 | 0.88 | 0.75 | 0.56 | 0.13 |
| | D | 0.82 | 0.82 | 0.79 | 0.77 | 0.59 |
| | E | 0.97 | 0.97 | 0.97 | 0.94 | 0.23 |

4.3.5 Genetic relatedness

Genetic relatedness between individuals of a group inhabiting an anemone was conducted using KINGROUP v2 (Konovalov et al. 2004). In this program, the method “kinship pairwise” (Goodnight and Queller 1999) was chosen to construct the coefficient of relatedness (r), which estimates patterns of kinship in natural populations. An r value less than zero means individuals from the same anemone are unrelated, and an r value greater

than zero means individuals within anemones are related. The present study analysed relatedness in groups of anemonefish resident in anemones from three populations: *A. ocellaris* from Barrang Lompo (n = 53; 17 groups), *A. ocellaris* from Samalona (n = 276; 83 groups), and *A. perideraion* from Barrang Lompo (n = 100; 35 groups). All groups which consist of one individual were excluded from the mean relatedness calculation. A one-sample *t*-test was used to test whether the probability that two individuals from the same group share an allele is no different from the probability that two individuals from the study population share an allele (Buston et al. 2007). The statistical analysis was conducted in STATISTICA 7 (Statsoft 2004).

4.4 Results

4.4.1 Summary statistics, Hardy-Weinberg equilibrium, and linkage disequilibrium

All markers used in the analysis were highly informative, as the average PIC value was 0.766 ± 0.161 (mean \pm SD) and ranged from 0.465 (locus 120) to 0.925 (locus Cf42) in the *A. ocellaris* populations, and 0.799 ± 0.154 (mean \pm SD) with a range of 0.601 (locus AC915) to 0.969 (locus Cf42) in the *A. perideraion* population. The number of alleles varied between five (locus 120) and 30 (loci Cf42 and AC137) in the *A. ocellaris* populations, and between five (loci AC915 and AC1578) to 57 (Cf42) in the *A. perideraion* population. The observed heterozygosity (H_o) ranged from 0.500 (120) to 0.977 (Cf29), and the expected heterozygosity (H_e) ranged from 0.539 (120) to 0.931 (Cf42) in the *A. ocellaris* populations. In the *A. perideraion* population, the H_o ranged from 0.267 (AC1578) to 0.952 (Cf42), and the H_e ranged from 0.653 (AC915) to 0.975 (Cf42). HWE exact tests indicated that loci AC1578 was not in HWE based on the probability test with $P < 0.01$ (Table 3). However, this loci was in HWE based on alternative hypothesis of heterozygote deficiency ($P = 0.087$). Therefore, this locus remained in the dataset for further analysis. In the *A. perideraion* population, HWE tests indicated two loci (Cf42 and AC1578)

with deviations from equilibrium. However, these loci remained in the dataset for further analysis as well. All loci combinations did not show significant linkage disequilibrium, indicating that all loci can be considered as independent.

Table 4.3: Summary statistics of *Amphiprion ocellaris* population dataset from (A) Barrang Lompo (N=88) and (B) Samalona (N = 276) over eight polymorphic microsatellite loci, and (C) *A. perideraion* population dataset from Barrang Lompo (N=105) over six polymorphic microsatellite loci. Observations include: name of the locus (Locus), allele size range (Allele), number of alleles (k), polymorphic information content (PIC), the observed (H_o) and expected (H_e) heterozygosity, and P values for Hardy-Weinberg exact test (Prob.= Probability test; H_1 = Heterozygote deficiency), significant values after sequential Bonferroni correction ($P < 0.01$) are indicated by an asterisk.

| Locus | Allele (bp) | k | PIC | H_o | H_e | Prob. | H_1 |
|----------|-------------|-----|-------|-------|-------|---------|---------|
| A | | | | | | | |
| Cf9 | 262 – 298 | 10 | 0.765 | 0.807 | 0.797 | 0.869 | 0.519 |
| Cf29 | 190 – 234 | 18 | 0.905 | 0.977 | 0.917 | 0.879 | 0.95 |
| Cf42 | 262 – 320 | 25 | 0.920 | 0.955 | 0.930 | 0.329 | 0.432 |
| 45 | 216 – 246 | 12 | 0.648 | 0.523 | 0.674 | <0.01* | 0.013 |
| 120 | 454 – 462 | 5 | 0.465 | 0.500 | 0.539 | 0.265 | 0.076 |
| AC137 | 256 – 322 | 20 | 0.912 | 0.920 | 0.923 | 0.679 | 0.192 |
| AC1578 | 250 – 264 | 8 | 0.755 | 0.727 | 0.792 | 0.117 | <0.001* |
| B | | | | | | | |
| Cf9 | 262 – 302 | 11 | 0.772 | 0.815 | 0.800 | 0.861 | 0.469 |
| Cf29 | 200 – 248 | 21 | 0.890 | 0.967 | 0.900 | 0.004* | 1 |
| Cf42 | 258 – 324 | 30 | 0.925 | 0.935 | 0.931 | 0.705 | 0.597 |
| 45 | 216 – 246 | 14 | 0.551 | 0.572 | 0.580 | 0.081 | 0.323 |
| 120 | 450 – 470 | 9 | 0.523 | 0.594 | 0.592 | 0.91 | 0.599 |
| AC137 | 250 – 328 | 30 | 0.920 | 0.913 | 0.926 | 0.334 | 0.031 |
| AC1578 | 250 – 266 | 9 | 0.781 | 0.783 | 0.810 | <0.001* | 0.087 |
| C | | | | | | | |
| Cf42 | 258 – 408 | 57 | 0.969 | 0.952 | 0.975 | <0.001* | <0.001* |
| 120 | 456 – 480 | 11 | 0.787 | 0.771 | 0.813 | 0.759 | 0.316 |
| AC137 | 276 – 336 | 24 | 0.925 | 0.790 | 0.934 | 0.014 | <0.001* |
| AC915 | 218 – 230 | 5 | 0.601 | 0.619 | 0.653 | 0.808 | 0.265 |
| AC1578 | 250 – 258 | 5 | 0.634 | 0.267 | 0.695 | <0.001* | <0.001* |

4.4.2 Self-recruitment

In Barrang Lompo, parentage analysis of 25 *A. ocellaris* juveniles and 31 potential parents sampled in 2008 assigned 12 juveniles to single parents (48 %) and one juvenile to a parent pair (4 %) (Table 4). Parentage analysis of 32 *A. ocellaris* juveniles and 31 potential parents that were collected at Barang Lompo in 2009 assigned 14 juveniles to single parents

(43.8 %) and zero to parent pairs (0 %). In total, the self-recruitment for *A. ocellaris* at Barrang Lompo in 2008 and 2009 was 47.4 %.

At Samalona, parentage assignment identified 68 for single parent (60.7 %) and five for parent pair (4.5 %) among 112 juveniles in 2009 as being progenies of 164 potential parents. For *A. perideraion* at Barrang Lompo, parentage analysis identified 28 for single parents (43.75 %) and two for parent pair (3.13 %) among 64 juveniles as being progenies of 41 potential parents. A total of 6 juveniles (19.4 %) in 2008 and of 6 juveniles in 2009 (19.4 %) from Barrang Lompo population were identified as being progenies of 164 potential parents in Samalona. While a total of 4 juveniles (10.3 %) in 2009 from Samalona population were identified as being progenies of 31 potential parents in Barrang Lompo.

Table 4.4: Self-recruitment of anemonefishes at Barrang Lompo and Samalona. At Barrang Lompo has two sampling periods (2008 and 2009) for *Amphiprion ocellaris* and one (2009) for *A. perideraion*, while Samalona has one sampling period (2009) for *A. ocellaris*. P = potential parent, J = juveniles.

| | Self-recruitment | |
|--|------------------|--------------|
| | Single parent | Parent pair |
| Barrang Lompo | | |
| <i>Amphiprion ocellaris</i> (2008, P = 31, J = 25) | 12/25 (48%) | 1/25 (4%) |
| <i>A. ocellaris</i> (2009, P=31, J=32) | 14/32 (43.8%) | 0/32 (0%) |
| <i>A. perideraion</i> (2009, P=41, J=64) | 28/64 (43.4%) | 2/64 (3.1%) |
| Samalona | | |
| <i>Amphiprion ocellaris</i> (2009, P=164, J=112) | 68/112 (60.7%) | 5/112 (4.5%) |
| Mix <i>A. ocellaris</i> | | |
| Samalona parents, Barrang Lompo juveniles 2008 | 6/31 (19.4%) | 1/31 (3.2%) |
| Samalona parents, Barrang Lompo juveniles 2009 | 6/31 (19.4%) | 0/31 (0%) |
| Barrang Lompo parents, Samalona juveniles | 4/39 (10.3%) | 0/39 (0%) |

4.4.3 Site fidelity

The present study investigated the site fidelity of juveniles to their natal site (Fig. 4.3), indicating how far larvae dispersed on an island scale. A relatively high site fidelity of juveniles to their origin site and low dispersal distances was revealed. Individuals of *A. ocellaris* and *A. perideraion* were leaving their native site in different proportions. However, most of them settled close to their origin site. The percentage of

juveniles of *A. ocellaris* that returned to their natal site in Barang Lompo was up to 44 %, while for *A. perideraion* up to 19 %. In the Samalona, the percentage of juveniles that returned to their origin site ranged from 8 % to 11 %.

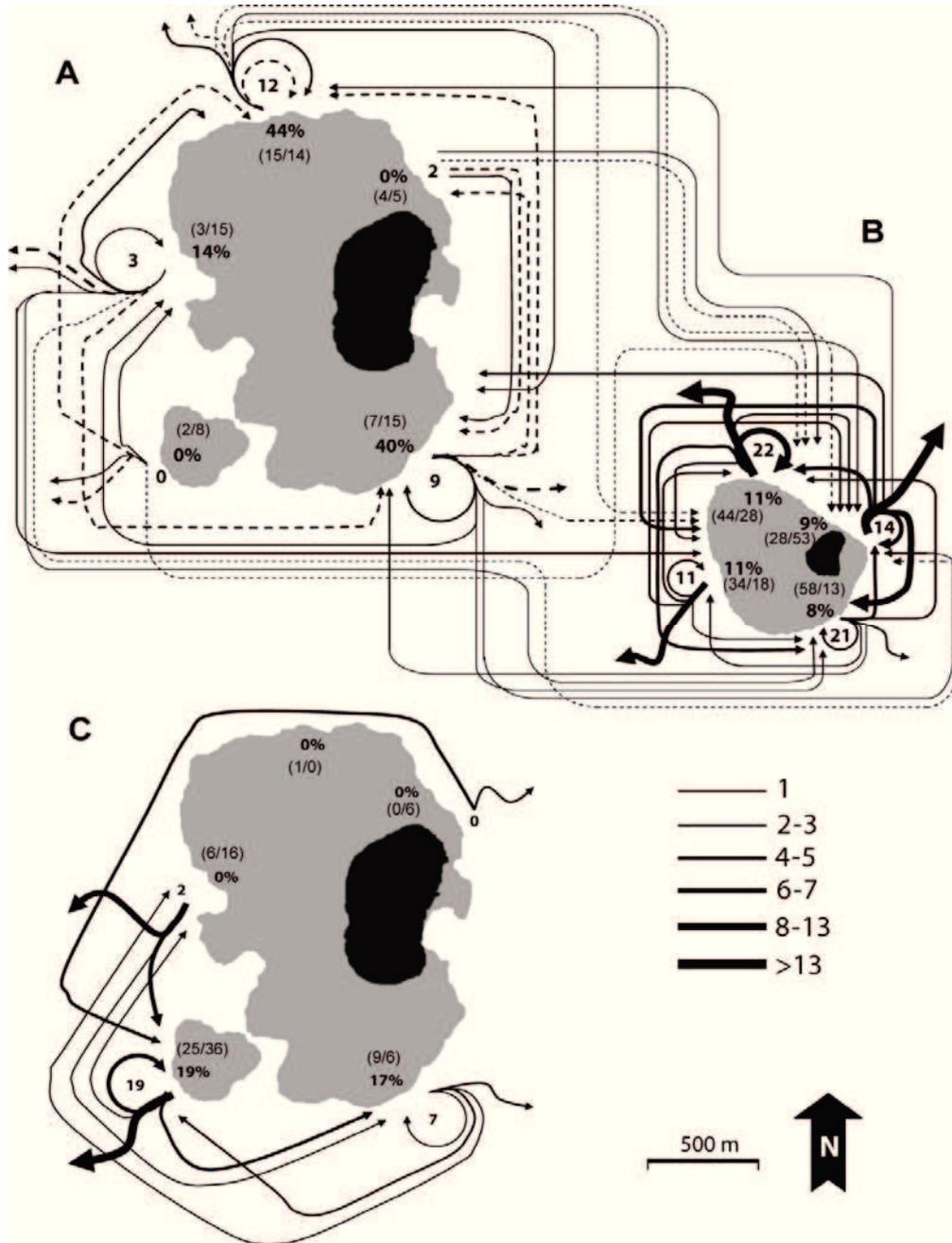


Fig. 4.2: Site fidelity of *Amphiprion ocellaris* at (A) Barrang Lompo in 2008 (line) and 2009 (dash); (B) *A. ocellaris* at Samalona in 2009. The two islands are not oriented to each other as shown here, see Fig.4.1 for detail; and (C) *A. perideraion* at Barrang Lompo in 2009. All juveniles in the analysis were identified as being the progenies of adults of the present study sites using DNA parentage analysis with the program FAMOZ. Numbers of juveniles returning to their potential natal site are shown at each site. Percentage at each sites shows percentage of individual that stayed at their potential natal site. In brackets are number of potential parent and juveniles. Black area: island; grey area: coral reef.

4.4.4 Genetic relatedness

The mean coefficient of relatedness of *Amphiprion ocellaris* individuals within groups at Barrang Lompo (mean \pm SD = 0.108 ± 0.162 , $n = 17$ groups) is significant (one-sample t -test: d.f. = 16, $t = 2.754$, $P = 0.014$, Fig. 4.3a). This shows a close relation of *A. ocellaris* individuals within groups at Barrang Lompo. This indicates that the probability that two individuals from the same group share an allele is not the same as the probability that two individuals from the study population share an allele. Conversely, the mean coefficient of relatedness of *A. ocellaris* individuals within groups at Samalona (mean \pm SD = 0.001 ± 0.114 , $n = 83$ groups) is not significant (one-sample t -test: d.f. = 82, $t = 0.062$, $P = 0.951$, Fig. 4.3b). The mean coefficient of relatedness of *A. perideraion* individuals within groups at Barrang Lompo (mean \pm SD = 0.051 ± 0.151 , $n = 35$ groups) is not significant (one-sample t -test: d.f. = 34, $t = 2.014$, $P = 0.052$, Fig. 4.3c). This shows that individuals of *A. ocellaris* in Samalona and *A. perideraion* at Barrang Lompo within groups are not closely related. It implies that the probability that two individuals from the same group share an allele is the same as the probability that two individuals from the study population share an allele.

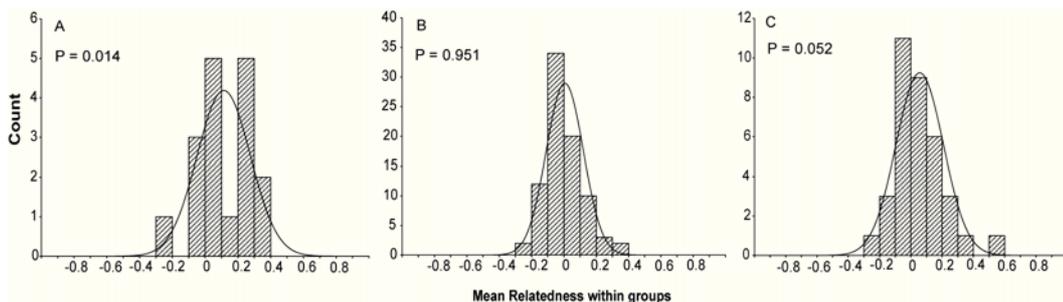


Fig. 4.3: Distribution of the average relatedness within group anemones: (A) *Amphiprion ocellaris* in Barrang Lompo (53 individuals, 17 groups; one-sample t -test: d.f. = 16, $t = 2.754$, $P = 0.014$), (B) *A. ocellaris* in Samalona (276 individuals, 83 groups; one-sample t -test: d.f. = 82, $t = 0.062$, $P = 0.951$), and (C) *A. perideraion* in Barrang Lompo (100 individuals, 35 groups; one-sample t -test: d.f. = 34, $t = 2.014$, $P = 0.052$).

4.5 Discussion

4.5.1 Self recruitment

The present study revealed high self-recruitment of anemone fish by highly polymorphic microsatellite DNA parentage analysis. Self-recruitment of *Amphiprion ocellaris* (60.7 %) at Samalona is higher than that reported from a previous study on the sibling species *A. percula* (Planes et al. 2009). The high self-recruitment in Samalona is in agreement with evidence of restricted gene flow revealed in the Spermonde Archipelago and across Indonesia (Timm and Kochzius 2008). The high self-recruitment might be triggered by sheltered environment within the mid-shelf of the Spermonde Archipelago, where the present study sites are located, compared to the outer-shelf of the archipelago which is strongly affected by Northwest Monsoon (Tomascik et al. 1997). The level of self-recruitment, found in Samalona, could play an important function in restocking and restoring the archipelago's populations (Lipcius et al. 2008).

The lower estimated self-recruitment in Barrang Lompo (*Amphiprion ocellaris* : 45.6 %; *A. perideraion*: 43.8 %) is possibly biased by long-term high fishing pressure (Erdmann 1995; Edinger et al. 1998; Chozin 2008). Due to that, parent anemonefish are probably removed by ornamental fishermen, deflating the estimate of self-recruitment.

The pelagic larval duration (PLD) varies from days to weeks in different species of coral reef fish (Wellington and Victor 1989) and thus may influence their dispersal distance. Due to pelagic dispersal of eggs and larvae, most marine species are considered as open populations (Caley et al. 1996), even though this is still under discussion (Cowen et al. 2000; Mora and Sale 2002). In an open marine population, fish larvae are assumed to be easily transported by predominant currents during their pelagic stage over long distances, facilitating high connectivity among populations (Roberts 1997). However, many recent studies using different methodologies and molecular markers estimated a high self-recruitment level in different marine fish species with different PLDs, suggesting low

dispersal leading to low connectivity among populations. For example an assignment test using microsatellite loci in *Tripterygion delaisi* (with 16–21 days PLD) (Carreras-Carbonell et al. 2007), otolith marking in *Pomacentrus amboinensis* (16–19 days) (Jones et al. 1999), tetracycline mass-marking and parentage analysis in *Amphiprion polymnus* (9–12 days) (Jones et al. 2005), and otolith microstructure and microchemistry analysis in *Sebastes melanops* (83–174 days) (Miller and Shanks 2004), and *Amphiprion ocellaris* (8–12 days) (this study). Therefore, it seems as if the dispersal of pelagic fish larvae may be more restricted and marine systems may not be as open as previously assumed.

4.5.2 Genetic relatedness and site fidelity

The analysis of the genetic relatedness of *Amphiprion ocellaris* at Barrang Lompo showed that individuals within group anemones are closely related, indicating that fish larvae remains in their origin site. This close relationship might be explained by several mechanisms. Many marine fishes have the ability to recognise their relatives in order to avoid inbreeding and competition (Ward and Hart 2003). However, due to a low abundance of anemones as a result of their removal for ornamental fishery happening at Barrang Lompo (Madduppa et al. under review), the pelagic larvae may find difficulties to avoid staying within their origin or a nearby anemones. Anemonefish larvae remain to the first anemone they encounter, without considering the number or identity of residents (Buston 2004). This close relatedness between individuals in an anemone may force inbreeding. Inbreeding has some consequences, such as deleterious alleles in the first generation because of strong breeding system in anemonefish (Fricke and Fricke 1977, Buston 2004), and reduces adaptive level of species (Wright 1932). Inbreeding in a population could affect decrease in the heterozygosity and increase in the homozygosity which decreases species fitness and could lead to mortality (Charlesworth and Charlesworth 1987). In contrast, unrelated individuals were identified within group anemones of *A. ocellaris* at Samalona and *A. perideraion* at

Barrang Lompo. These results agree with findings from congeneric individuals of *A. percula* (Buston et al. 2007) and *Dascyllus aruanus* (Buston et al. 2009), forming groups consisting of unrelated individuals, which have the implication that larvae leave their native sites.

This study shows that anemonefish larvae leave their origin anemone, but most of them do not venture far (less than 2 km) from their origin sites (see Fig.3 for details). Both islands' populations show that the different groups were connected by dispersal of larvae among the groups within the island populations. Between Barrang Lompo and Samalona population, which is separated in a distance about 7.5 km, were connected by juveniles' exchange range from 10 to 19 %. Larval dispersal in marine organisms can vary from less than 1 km to 100s km (Swearer et al. 2002; Cowen et al. 2006; Saenz-Agudelo et al. 2009; Salas et al. 2010; Saenz-Agudelo et al. 2011). A study of self-recruitment in *A. polymnus* discovered that even though no individuals settled into the same anemone as their parents, most of them settled close to their origin group (Jones et al. 2005), which is in concordance with the findings in *A. ocellaris* and *A. perideraion* of the current study. With a proper estimation of connectivity and degree of self-recruitment in marine populations can improve the design of marine reserves. For example, MPA networks can serve to maintain local populations both by self-recruitment and through larval dispersal from other reserves (Planes et al. 2009).

The connectivity within and among populations might be also influenced by the behaviour of the planktonic larvae itself as well as and its parents. Planktonic larvae of marine fish are assumed to be able to recognise and return to their parent habitat. It was shown that the capability to swim and orientate, as well as vertical positioning capabilities of the demersal fish *Amblyglyphidodon curacao* can strongly influence dispersal results (Leis et al. 2007). The early inception of the active larval movement is important to mediate the dispersal potential (Cowen et al. 2006). Olfactory sensing might also limit larval movement (Gerlach et al. 2007). It was shown that *Amphiprion ocellaris* imprints

itself to its species-specific host sea anemone using olfactory cues, which are genetically inclined towards olfactory recognition of their host anemone (Arvedlund and Nielsen 1996). In order to facilitate retention reef fish may use odour recognition and preference by larval fish (Atema et al. 2002). In addition, the connectivity within and among populations in anemone fish could occur considering their relatively short PLD of about 8–12 days. However, exploitation of species, determined by decreased population density and body size reduction (Polunin and Roberts 1993; Birkeland and Dayton 2005; Madduppa et al. under review), could reduce larval abundance and shrink the dispersal kernel and effective connectivity distances (Steneck 2006).

4.5.3 Implications for management and conservation

Anemone fish have been exploited for marine ornamental fishery and traded globally for many years. Most marine ornamental fish species are collected from the wild, with Indonesia and the Philippines as the major exporters (Wabnitz et al. 2003). High fishing pressure caused a decrease in the abundance of the exploited species (Hsieh et al. 2006). In order to avoid overexploitation and to reduce the pressure on natural populations, some efforts have been made to rear ornamental fish species (Danilowicz and Brown 1992; Ogawa and Brown 2001; Johnston et al. 2003). Anemone fish such *A. ocellaris* have been successfully bred in captivity (Madhu et al. 2006), which is potentially a good solution to meet the high demand. However, mariculture needs comprehensive knowledge of the biology of the reared fish species and is expensive (Bardach et al. 1972; Ogawa and Brown 2001; Tucker 2000). Therefore, it seems that mariculture would be difficult to be implemented for many ornamental species and to many regions. In the Spermonde Archipelago, *A. ocellaris* is the most collected marine ornamental fish species and this fishery has impacted the population of this targeted species (Madduppa et al. submitted). However, there is no quota for anemonefish species in Indonesia so far.

The two focal species (*A. ocellaris* and *A. perideraion*) have not yet been included in the CITES list (Convention on International Trade in Endangered Species of Wild Fauna and Flora), which aims to prevent overexploitation by international trade (CITES 2011). Considering the high level of exploitation, a proper management plan and conservation strategy should be implemented for this species in Spermonde Archipelago (Madduppa et al. under review). Implementation of MPAs as a tool to manage fisheries and marine biodiversity has been used to protect coral reefs from overexploitation, increase fish populations, restore ecosystem health, and prevent local extinctions (Agardy 1994; Bohnsack 1998; Gonzalez et al. 1998; Botsford et al. 2003). However, determining the optimal size of self-sustaining MPAs is essential for promoting population persistence (Sala et al. 2002; Gell and Roberts 2003; Palumbi 2004; Sale et al. 2005).

The high amount of self-recruitment of *Amphiprion ocellaris* and *A. perideraion* found in the current study gives valuable information for management and conservation strategies within the region. High levels of self-recruitment imply that the populations are more vulnerable to fishing activity (Thorrold et al. 2001). While further studies on other marine organisms are needed, the current study suggests that single marine protected areas (MPAs) are not suitable as sources for the replenishment of exploited populations. Small MPAs, preferably on every island/reef, should ensure that a part of the population is protected to allow for replenishment by self-recruitment. Such small MPAs are suitable for the archipelago consisting of over 150 islands, most of which are relatively small and many are inhabited. Therefore, a number of small MPAs within the region could ensure the sustainability of the anemone fish populations and prevent them from being depleted. In addition, the population size should be extensively estimated by underwater visual census in order to establish appropriate catch quotas.

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Declaration

Gemäß §6 der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14. März 2007 versichere ich, dass:

1. die Arbeit ohne unerlaubte fremde Hilfe angefertigt wurde
2. keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden
3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht wurden

Bremen, March 2012

Hawis H. Madduppa

Glossary

| | |
|--------------------------------|---|
| Allele | A variant form of a gene. In a diploid cell there are two alleles of every gene (one inherited from each parent, although they could be identical). Within a population there may be many alleles of a gene. Alleles are symbolized with a capital letter to denote dominance, and lower case for recessive. In heterozygotes with co-dominant alleles, both are expressed. |
| Allele frequency | The relative number of copies of an allele in a population, expressed as a proportion of the total number of copies of all alleles at a given locus in a population. |
| Allelic diversity | The variation in alleles in a population |
| <i>Amphiprion</i> | Fish genus containing 27 species, all of which are symbiotic with sea anemones |
| Assignment test | Statistical approach to place an individual in the population to which it is, on average, most closely related |
| Base pair | Matched bases in a DNA molecule, with one on each of the two complementary strands. Base pairs in DNA are A-T or G-C. |
| Bottleneck | A large reduction population size, often followed by a recovery |
| Density | The number of individuals per unit of area |
| DNA | Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms |
| Electrophoresis | Separation molecules through a solid matrix (usually a gel) by application of an electric field |
| Expected heterozygosity | The estimated fraction of all individuals that would be heterozygous for any randomly chosen locus. It differs from H_o because it is a prediction based on the known allele frequency from a sample of individuals |
| Gene | A defined sequence of DNA that is transcribed into RNA |
| Gene flow | Transfer of genes from one population to another of the same species, as by migration or the dispersal |
| Genetic diversity | The amount of genetic variation present in a population, often quantified as expected heterozygosity or allelic richness (codominant markers), and gene or haplotype diversity (dominant or haploid markers) |
| Genetic drift | Changes in allele frequency that occur by chance, essentially as a random sampling of available gametes each generation |
| Genetic relatedness | A quantitative estimate of the proportion of genes, shared between the genomes of any two individuals, groups or populations |
| Genetic marker | A DNA sequence used to identify a particular location (locus) on a particular chromosome |
| Genotype | The genetic constitution of an organism at one, many or all genetic loci |
| <i>Heteractis</i> | Genus containing four species of sea anemones, all of which may host anemonefish |
| HWE | Hardy-Weinberg equilibrium – the proportions of homozygotes and heterozygotes expected in a large, randomly mating (panmictic) population when overall allele frequency are known. Assuming no migration, mutation or selection |
| Heterozygosity | The observed or expected (under HWE) proportion of heterozygotes in a population |
| Heterozygote | Diploid individual with two different alleles at a genetic locus |
| Homozygote | Diploid individual with two identical alleles at a genetic locus |
| Host | One of the partners in a symbiosis, generally the larger one (thus, the |

| | |
|--------------------------------|---|
| | sea anemones that are subject of this dissertation are host to fish) |
| Inbreeding | The breeding together of close relatives |
| Inbreeding depression | Fitness reduction consequent upon interbreeding by close relatives |
| ITF | Indonesian Trough Flow, |
| Larva (plural larvae) | A development stage that hatches from an egg and that typically lives in a different environment, looks entirely different, and eats different food from the adult of the species, oceanic larvae are generally very small, and are planktonic |
| Linkage equilibrium | Situation, promoted by recombination, in which genetic loci segregate independently of one another during reproduction. Disequilibrium occurs when alleles at two loci segregate together, for example, when they are physically close on the same chromosome |
| Locus | A defined sequence of DNA on a chromosome. May or may not be a gene. |
| Marine ornamental trade | The trade on marine organisms such as coral reef fish, fish, invertebrate for aquarium hobbyist |
| Microsatellite | Genetic locus with a simple sequence (usually di, tri or tetranucleotide) repeated multiple times. |
| Monomorphic | A locus with a single allele, thus displaying no genetic variation |
| Mutation | Alteration of the nucleotide sequence in DNA |
| Obligate symbiosis | A relationship in which one partner must live with another in order to survive, except for a brief planktonic larval stage, anemonefishes are obligate symbionts of sea anemones |
| Observed heterozygosity | Number of heterozygotes at a locus per total number of individuals surveyed |
| Over exploitation | exploitation to the point of diminishing returns |
| Null alleles | Alleles that fail to amplify by the PCR, commonly associated with microsatellites and mutations in their flanking (primer-binding) regions |
| Panmictic | (= Panmixia) A population with random mating among all the individuals present |
| PCR | Polymerase Chain reaction, a method for amplifying large quantities of a DNA sequence using oligonucleotide primers and a thermostable DNA polymerase |
| Population genetics | The branch of science that deals with the statistical analysis of the inheritance and prevalence of genes in populations. |
| Population connectivity | The linking of distinct populations by individual dispersal or migration. |
| Primer | Short oligonucleotide (typically 15-15 nucleotides long) complementary to a DNA sequence and which can be used in PCR amplifications |
| Sea anemone | Any of numerous flowerlike marine coelenterates of the class Anthozoa, having a flexible cylindrical body and tentacles surrounding a central mouth. |
| Self-recruitment | number of larvae returning to and settling in their natal population |
| Standard deviation | (= SD) Square root of the variance |
| Standard error | (= SE) Typically standard deviation of sample means or differences |
| <i>Stichodactyla</i> | Genus containing five species of sea anemones, three of which may host anemonefish |

Sources:

Beebe T, Rowe G (2008) An introduction to Molecular Ecology. Second Edition. Oxford University Press

www.expertglossary.com

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Abbreviations

| | |
|--------------------|---|
| °C | degree Celcius |
| µl | microlitre |
| µmol | micromol |
| Ann. | annealing temperature |
| bp | basepairs |
| COREMAP | Coral Reef Rehabilitation and Management Project |
| CITES | Convention on International Trade in Endangered Species of Wild Fauna and Flora |
| ddH ₂ O | double distilled Water |
| DNA | deoxyribonucleic acid |
| dNTPs | deoxynucleotidetriphosphates |
| FAM | Carboxyfluorescein |
| GBR | Great Barrier Reef |
| GPS | Global Positioning System |
| D | mean gene diversities over all loci |
| HD. | Heterozygote deficit |
| HE | High Exploitation |
| He | Expected Heterozygosity |
| Ho | Observed Heterozygosity |
| HEX | Hexachlorofluorescein phosphoramidite |
| HWE | Hardy Weinberg Equilibrium |
| ITF | Indonesian Through Flow |
| km | kilometre |
| LE | Low Exploitation |
| LOD | The log-likelihood ratios or logarithm of the odds |
| m | metre |
| MgCl ₂ | Magnesium chloride |
| min | minutes |
| mM | micro Molar |
| mm | milli metre |
| ML | Maximum Likelihood |
| MPA | Marine Protected Area |
| Na | number of alleles |
| ng | nano gram |
| PCR | Polymorphic Chain Reaction |
| PIC | Polymorphic Information Content |
| PLD | Pelagic Larva Duration |
| s | seconds |
| SR | Self-Recruitment |
| SPICE | Science for Protection Indonesian Coastal Ecosystem |
| WDPA | World Database Protected Area |

Indonesia's coral reefs have been considered as the centre of coral triangle due to high number of coral species and reef fishes. While these reef resources sustain the livelihoods of millions of people, they are also among the most threatened by anthropogenic activities, including pressure from marine ornamental trade. Spermonde Archipelago is one of the main collection sites for marine ornamental trade in Indonesia. Many coral reef fishes are currently collected for the aquarium trade, of which the clown anemonefish "Nemo" *Amphiprion ocellaris* is the most popular species. The trade is ongoing, unfortunately the total catch and the potential impact of high exploitation rates are not known. Additionally, no proper management and conservation efforts exist in Indonesia to protect this species. These situations will potentially lead to the degradation of marine biodiversity. Thus, a proper management, including the implementation and effective enforcement such as marine protected areas (MPAs) is needed to avoid overexploitation and depletion of this commercially harvested fish species. The connectivity of populations and the extent of self-recruitment are important aspects for the management of living resources and designing and improving MPAs.